Supplementary Information

"Intestinal crypt homeostasis revealed at single stem cell level by *in vivo* live-imaging" by Ritsma et al.

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Supplementary Notes

Background: Neutral drift model of intestinal stem cell self-renewal

In previous studies, attempts were made to resolve the dynamics of intestinal stem cells in mice using the results of two medium- and long-term lineage tracing assays based on inducible genetic labelling. The first of these studies made use of a ubiquitous promoter (Ah*cre*^{ERt}) to monitor the size distribution of a statistical ensemble of single-cell derived clones over time'[8]. By characterising the size of the underlying stem cell pool by the width of migration streams of differentiated progeny on the walls of the villi, it was shown that the progressive drift towards clonal fixation of individual crypts was preceded by a regime in which the clone size distribution showed a characteristic scaling dependence. The particular behaviour represented a hallmark of one-dimensional 'neutral drift dynamics', and its observation provided strong evidence that the long-term maintenance of the intestinal epithelium involved the stochastic loss and replacement of neighbouring stem cells. Further, since the clonal migration streams of differentiated cells on the villi remained cohesive (i.e. non-fragmented), it was evident that cell or clonal migration through the apex of the crypt base must be rather infrequent.

Altogether, this behaviour motivated a simple modelling scheme in which an equipotent population of intestinal stem cells are organised as a one-dimensional 'collar' or annulus around the stem cell niche at the crypt base. As stem cells divide, some are displaced from the niche and progressively loose stemness. Through this process of stem cell loss and replacement, there is a gradual depletion in clonal diversity allied with an increase in the size of surviving clones. As well as providing a basis to understand the scaling behaviour of the clone size distribution, this model also described quantitatively the dynamics of clone fixation, i.e. the gradual drift of the crypt towards monoclonality.

Although this simple model provided insight into stem cell dynamics, several important questions were left open. In particular, by making use of a ubiquitous promoter, the molecular (and therefore the spatial) identity of the stem cell compartment could not be resolved. Moreover, since the model dynamics was largely dependent only on the ratio of the stem cell loss/replacement rate to the square of the effective stem cell number per crypt, λ/N_{stem}^2, the individual parameters remained unspecified, i.e. for the same ratio, a large stem cell number, N_{stem}, would translate to a larger

loss/replacement rate, etc. (later, we will return to discuss more precisely what is meant by 'effective' stem cell number).

In a parallel investigation, the behaviour of individually labelled $Lgr5^+$ CBC cells was analysed using the same Confetti-construct exploited in the current study[7]. Using this approach, it was possible to score a statistical ensemble of clone sizes at single cell resolution over a range of time points, and disaggregated according to their expression of Lgr5. By exploiting the same one-dimensional modelling scheme, a quantitative fit to the data suggested that the intestinal stem cell population perfectly overlaps with the pool of Lgr5 expressing cells. In short, on the basis of this analysis, it was proposed that the $Lgr5^+$ population constitutes a single equipotent stem cell pool in which the long-term survival probability of all Lgr5 expressing cells was equal.

However, on the basis of the current *in vivo* live-imaging study, we find that this conclusion is incorrect. Although all Lgr5 expressing cells retain long-term survival potential, the results of *in vivo* live-imaging combined with quantitative analysis of lineage tracing data shows that stem cells experience a positional bias towards self-renewal or loss according to their position within the niche. In the following supplementary notes, we set out in more detail the theoretical basis and practical implementation of a revised modelling scheme to address the lineage data, and return later to discuss why the previous single-cell resolution study, based on an analysis of static clonal fate data, failed to discern a short-term bias in stem cell self-renewal potential.

Lessons from the clonal fate data

To resolve potential short-term dynamics of stem cells and address their self-renewal potential, it is essential to address clonal evolution at the earliest time points following labelling, where non-universal (i.e. non-scaling) features of the clone dynamics can impact. It is at these early times that the reliability of a static inducible genetic labelling assay is most severely challenged: In particular, the transient and potentially deleterious effect of the Cre recombinase or drug inducing agent may influence cell behaviour. Second, the potential variability of cell induction frequency with position in the cell cycle may lead to the synchronisation of marked cells, an effect exacerbated by the inevitable synchrony in the timing of consecutive cell divisions for such rapidly dividing cells. Third, the persistence of *cre* activity may result in a prolonged period of induction of Confetti-expression, confusing early time measurements. Fourth, the delay in the accumulation of fluorescent protein may bias the visibility of clones at early times in an uncertain and potentially non-representative manner.

Fortunately, by imposing a delay between drug administration and clone detection, the live-imaging assay provides the means to circumvent these difficulties, providing a largely unbiased measure of the early time dynamics from which any bias in the short-term survival potential of stem cells can be resolved. Moreover, by tracing the *same* clone over time, an 'initial' cell configuration of a clone (its size and position within the stem cell niche) can always been defined at any time within its history. As a result, an effective induction time can be assigned from the lineage itself allowing sub-lineages of the parent lineage to be used to consolidate the data. For example, a clone that progresses through the sequence, say, (border, centre)=(1,1) at day 0, (0,1) at day 1, (2,1) at day 2 and (2,3) at day 3 can be used to extract the single centre-cell derived lineage: (0,1) at day 0, (2,1) at day 1 and (2,3) at day 2, etc. Therefore, since the actual time at which cells become marked is irrelevant, transient effects due to *cre* activity, etc., can be further mitigated by extending the delay (beyond the three day base line) between drug administration and the first measurement of clone size and composition.

From live-imaging, a total of 80 lineages were scored over a time window of 3 to 7 or 8 days following Tamoxifen administration. For each clone, the composition was recorded according to their cell content, disaggregated across the first five rows of the crypt (row 0 to +4) (Fig. 1b). The fourth row marks the boundary separating cells that are high in Lgr5 content from those that are negative, as assessed by GFP expression. Moreover, it also defines the extent of the Paneth cell region, the putative niche environment for stem cells of the small intestine. Since the TA cell component of clones could not be scored in detail, once all cells in the clone were depleted from the stem cell niche, it was deemed to have been lost and not traced further.

To further consolidate the live-imaging data, we associated CBC cells between rows 0 to +2 with the central region, and cells in rows +3 and +4 with the border region as detailed in Fig. 1b, Extended Data Fig. 4 and the main text. From multiphoton microscopy, it is evident that the border region may contain both stem and TA cells. Since it was difficult to quantify individual TA cells, we did not attempt to score quantitatively the size of this compartment within clones, nor therefore did we attempt to address cell dynamics within this compartment. Furthermore, although we acknowledge the existence of slow-cycling Lgr5^{+} cells in the crypt base region[15], we did not attempt to account for the potential impact of this population on the dynamics of cells in the niche region, nor did we assess directly their contribution to clone size. As just 1 of the 28 clones containing a single marked CBC cell at the start of imaging remained single after 2 days of tracing, we believe that the impact of this population on the interpretation of the clonal fate data is small. Equally, we cannot rule out the possibility that these quiescent cells are generated in the niche region through the division and

differentiation of the actively dividing confetti labelled cells. We note, however, that the origin and dynamics of this population will be of great interest in further dissecting the lineage potential and function of the Lgr5 expressing crypt base cells in future studies.

Before attempting to develop a revised modelling scheme, it is first useful to consider the qualitative behaviour of clones derived from different regions of the crypt. From the 80 lineages, we can extract 11 and 21 sublineages (as defined above) that derive from single cells in the central and border region, respectively. Referring to Fig. 2a, while the average number of labelled central cells in clones derived from a central cell remains roughly constant over the 3-day time course, consistent with their 'self-renewal', the number of crypt border cells derived from those clones increases approximately linearly over the same period, rising to an average of around 2 by day 3. However, the self-renewal of the labelled central compartment is associated with a progressive decline in the number of 'surviving' (non-expelled) clones (defined as clones containing at least one central CBC cell) allied with an increase in the size of these survivors (Fig. 2c). By contrast, although clones derived from single border cells also appear to approximately maintain their number over the 3 day time course, they give rise to a relatively small, but statistically significant, number of central cells over the same period (Fig. 2a). At the same time, the number of surviving border clones (defined as ones containing at least one border cell) diminishes over time (Fig. 2b).

Taken together, these observations impose important constraints on the dynamics of CBC cells. First, the progressive increase in the number of border cells following the clonal marking of a central cell in a homeostatic tissue suggests that the latter typically outcompetes the former for survival, i.e. central CBC cells typically expel border cells out of the stem cell niche (into the TA region). Moreover, the progressive reduction in the number of surviving central cell-derived clones suggest that cells within this layer compete with each other for niche space, consistent with the inferred neutrality of clonal dynamics at longer times implied by the previous lineage tracing studies. However, significantly, the acquisition of central cells from border-derived clones suggests that cells are able to interchange position between the central and border regions. These results indicate that cells in the border region are temporarily biased or 'primed' for displacement from the niche compartment and loss of stemness, while those at the central region are biased towards renewal. However, this priming is short-term: cells that transit between compartments may switch the direction of bias.

Based on these findings, and inspired by the success of the one-dimensional neutral drift dynamics model in defining the longer-term clonal behaviour in previous studies, we turned to the

development of a biophysical modelling scheme that can capture the cellular organisation and the effects of fate priming by position on the short and long-term clonal dynamics.

Biophysical model of intestinal stem cell dynamics

Based on qualitative insights from the live-imaging data, in the following, we propose that the differentiation of stem cells is associated with their displacement from the niche region, promoted by the rearrangement of cells in the central and border region following cell division. In this sense, all stem cell divisions are considered intrinsically symmetric, with the fate of daughters assigned subsequently through competition for niche access.

To make the model concrete, we specified the spatial organisation of stem cells within the niche, and the rules governing their fate behaviour. To this end, we proposed that stem cells are organized in a periodic quasi-one-dimensional lattice with two rows of cells representing the central and border regions of the stem cell niche, respectively. Note that this arrangement is chosen to be consistent with the apparent inability of stem cells to passage through the apex of the crypt, as reported in previous studies. Based on the observed coordination of Lgr5$^+$ CBC cells at the crypt base, we further proposed that central stem cells lie in contact with 4 neighbours, two in the central and two in the border region (Fig. 3a). To account for the mixed composition of cells in the border region (as evidenced by variability in GFP expression, and the expansion in the average size of the central cell-derived clones in the border region), we proposed that the differentiation of border stem cells involves the progression through a border TA cell compartment (i.e. rows +3 to +4). Finally, based on the observed frequency of GFP expressing cells in the central and border region, we supposed that both rows (central and border) host some 8 Lgr5$^+$ stem cells each (for details, see Fig. 3a).

In this scheme, to keep the size of the stem cell compartment constant, a stem cell division in the niche region must be correlated with the transfer of a border stem cell to a border TA cell through its displacement from the niche and attendant loss of stemness. This transfer is in turn accompanied by the displacement of a border TA cell into row +5. To this end, we can envisage at least five possible pathways to turnover: First, the division of a border cell may lead to an ‘asymmetric fate’ outcome in which one cell remains in contact with the Paneth cell niche and another is displaced into the border TA cell compartment leading to loss of stemness (Fig. 3a). Second, the division of a border cell may lead to a ‘symmetric fate’ outcome in which one of the daughters displaces a neighbouring border stem cell from the niche leading to its transfer into a border TA cell, which in turn displaces a border TA cell into row +5 (Fig. 3a). At the level of the border stem cell compartment, such a process mimics

the one-dimensional scheme developed in previous studies to address static clonal fate data. Third, the division of a border cell can lead to the downwards transfer of a daughter cell into the central region, displacing a central stem cell into the border region and leading in turn to the sequential displacement of another border stem cell out of the niche and into the border TA cell compartment (Fig. 3a). Such a process is necessary to facilitate the observed 'downwards' transfer of stem cells from the border to the central compartment of the niche. Fourth, following division of a central stem cell, a daughter may displace a border stem cell from the niche into the border TA cell compartment (Fig. 3a). Finally, a central stem cell division may lead to the displacement of a neighbouring central stem cell into the border region leading in turn to the displacement of a border stem cell into the border TA cell compartment (Fig. 3a).

With this definition, the long-term behaviour of the model is compatible with the known properties of the long-term clonal fate data. In particular, in common with the earlier model based on a strictly one-dimensional neutral drift dynamics, it follows that stem cell loss through detachment from the niche is locally correlated with replacement through division. Moreover, once a clone locally spans the central and border compartment, its further evolution will converge rapidly onto the (experimentally-observed) one-dimensional neutral drift dynamics, characteristic of a single equipotent population, leading to scaling behaviour and the characteristic drift of the crypt towards monoclonality.

Although one may envisage further refinements of the two-layer model above, involving for example the cyclic rearrangement of neighbouring stem cells in the central and border regions, such effects can be captured in full through the sequential steps associated with the dynamics above. Moreover, additional revisions of the model to accommodate spatial irregularity of the cellular organisation would further complicate the analysis without adding significant new insight.

Even within this simplified scheme, clonal dynamics are specified by no less than five adjustable parameters – the transfer rate, of TA cells from the border region away from the niche (rows +5 and above), λ, and the four independent probabilities defining the relative frequencies of the five 'channels' of replacement, P_b, P_{bb}, P_{bc}, P_{cb}, $P_{cc}=1-P_b-P_{bb}-P_{bc}-P_{cc}$ (see Fig. 3a for details), i.e. P_b represents the probability that the transfer involves the asymmetric fate outcome of a border stem cell, etc. Fortunately, combinations of these parameters can be inferred, or at least heavily constrained, from independent measurements of the clonal evolution.

First, since the net rate of transfer of cells from the border to the central region is relatively low (as compared with the corresponding rate of accumulation of border cells from central cell-derived

clones – see Fig. 2), to a first approximation it can be neglected altogether (i.e. we can begin with the assumption that P_{bc}=0). In this approximation, the central stem cell compartment can be treated as effectively 'closed' and perfectly self-renewing, with a dynamics formally equivalent to that of the original, strictly one-dimensional, model. The effective stem cell loss/replacement rate is then fixed by the product, $P_{cc}\lambda$, and the effective size of the self-renewing population, N_{stem}, is defined approximately by the number of Lgr5^{+} cells central CBC cells, estimated from GFP expression at around 8. At the same time, from the earlier analysis of scaling behaviour of clone sizes at intermediate times, and the progression to crypt fixation, previous studies have fixed the ratio $P_{cc}\lambda/N_{stem}^2$ at around 0.025±0.003/week,[8] from which we conclude that $P_{cc}\lambda$=0.23±0.05 per day. Here, the large error bar is dominated by the uncertainty in the estimate of N_{stem} – the contribution from downwards migration of stem cells, currently neglected, can elevate the true value of N_{stem}, while corrections due to Lgr5^{+} label-retaining progenitor cells at the stem cell niche may lower its value.

To set the remaining parameters, we must now take into account the flow of cells between the border and central region. From a fit of the initial growth rate of the average border cell content of central cell-derived clones (Fig 2a), it follows that $(P_{cc}+P_{cb}+P_{bc})\lambda$=0.7±0.2 per day. Similarly, from a fit of the initial growth rate of the average central cell content of border cell-derived clones (Fig 2a), it follows that $P_{bc}\lambda$=0.2±0.05 per day. Altogether, this suggests that the three rate constants, $P_{cc}\lambda$, $P_{cb}\lambda$, and $P_{bc}\lambda$ are all similar in magnitude and set at around 0.2-0.25 per day. These estimates are consistent with a net central stem cell division rate of $(P_{cc}+P_{cb})\lambda$=0.5 per day.

As well as defining the short-term properties of the central crypt region, the two rate constants, $P_{cc}\lambda$ and $P_{cb}\lambda$, largely fix the medium and long-term clonal evolution and the drift dynamics to monoclonality (see below). Moreover, if we interpret the transfer of cells from the central to the border region as a signature of commitment to differentiation – consistent with neglecting the reverse transfer of stem cells altogether, P_{bc}=0 – then we would deduce that some 50% of central stem cell divisions lead to stem cell loss/replacement while the remaining 50% lead to asymmetrical fate outcome.

Although it is more difficult to disentangle P_b and P_{bb}, to estimate the overall loss/replacement rate of differentiating border cells, we may note that the sum $(P_b+P_{bb}+P_{bc})\lambda$ includes all possible border cell divisions and therefore must translate to the average border cell division rate, estimated from assays based on the incorporation of thymidine analogues at around once per day.

Taken together, these four conditions heavily constrain the five model parameters, leaving only one adjustable parameter to fix the short-term clonal fate data. To fit this parameter – effectively

the ratio P_{bb}/P_b – we must obtain an estimate for the evolution of clone size probabilities over time. For the one-dimensional model, the probability distribution could be inferred analytically from the solution of the corresponding Master equation[8]. In the present case, the model is sufficiently complex that a formal analytic solution is unavailable. However, the dynamics can be straightforwardly resolved numerically through stochastic simulation.

Taking, for simplicity, the timing between consecutive cell divisions to be statistically uncorrelated, from the numerical simulation we obtain satisfactory fits to the short-time clonal fate data with λ=1.6 per day, P_b=0.5, P_{bb}=0.05, and P_{bc}=P_{cb}=P_{cc}=0.15, i.e. the cell division rate of border cells is given by $\Delta=(P_b+P_{bb}+P_{bc})\lambda$=1.1 per day, a figure close to that estimated from BrdU incorporation in the stem cell niche region[31]. In particular, referring to Figure 2a-c, Figure 3b and Extended Data Figure 7, we obtained favourable fits both to the average clone size dependences and survival probabilities as well as the distribution of clone sizes, disaggregated by cell position. Here, to estimate the confidence in the inferred parameters, we have shown the expected statistical variation of the model prediction (via the standard deviation) for the given cohort size of clones obtained from live-imaging.

Furthermore, with these parameters in hand, we can make use of the medium and long-term clonal fate data from pulse-labelling to further challenge the model. In particular, applied to the ensemble of clonal fate data from Snippert *et al.*[7], taken at 7 and 14 days post-induction, and allowing for a one day time offset due to the delayed action of the Tamoxifen on induction, we find that the model predictions agree well with the measured clone size distribution (Extended Data Fig. 8). Note that, although the model involves a relatively intricate short-term dynamics, which is sensitive to the spatial position of the induced stem cells, as mentioned above, once a surviving clone has expanded sufficiently to span the central and border layer in a representative manner, the resulting time-evolution will converge rapidly onto the dynamics of the one-dimensional (single-layer) system, leading in turn to the hallmark scaling behaviour of the clone size distribution visible in the clonal fate data at 14 days post-induction (Extended Data Fig. 8). Finally, applied to the study of the variation in the percentage fraction of fixed crypts over time, we also find that the model provides good agreement with the observed long-term clonal fate data (Extended Data Fig. 9, cf. measurements of Lopez-Garcia *et al.*[8]), providing an additional check on the validity of the modelling scheme.

Before concluding this section, it is instructive to reflect on the apparent contradiction between the current findings and the results of the earlier investigation based on the analysis of static clonal fate data by Snippert *et al.*[7]. Under the conditions of long-term steady-state turnover, *any* quasi-one-dimensional dynamics involving stem cell loss and replacement spanning more than one 'row' of

stem cells will converge over time onto the same scaling behaviour as that predicted by a strictly one-dimensional modelling scheme[24]. As discussed above, once a clone has expanded to span the rows in a representative manner, a process that takes place in a time comparable to the transfer of cells between rows, its further evolution will converge onto the dynamics of a strictly one-dimensional neutral drift model, characterised by an equipotent population, with an effective stem cell number, N_{stem}, which translates to the linear size of the compartment. Once in this limit, the clone size distribution becomes dependent only on the ratio of the loss/replacement rate to the square of the effective stem cell number, $\lambda/N_{\mathrm{stem}}^2$. As such, the universal scaling regime associated with the intermediate-time clone dynamics does not provide the means to resolve potential heterogeneity within the intestinal stem cell compartment. To resolve such factors, it is necessary to address clonal evolution at the earliest time points post-induction, where non-universal (i.e. non-scaling) features of the dynamics can impact. With just two rows (the border and central region), the time scale to 'equilibration' and scaling takes place within 2 to 3 days demanding clonal fate data at yet earlier times post-induction. Unfortunately, it is at these early times that the reliability of a static inducible genetic labelling assay is most strongly challenged. Although the model of a strictly one-dimensional dynamics implied by the simplified modelling scheme indeed provided a good agreement with the lineage tracing data at all times, including the earliest 2 day time point, the robust nature of the scaling dependence allowed the short-term heterogeneity to go unrecognized.

What do these findings imply about the range and identity of the intestinal stem cell compartment? First, the transfer of crypt base CBC cells between the border and central region shows that cells in the border region have not yet made the commitment to differentiation. Rather, the bias of border stem cells towards differentiation is a reflection of their spatial position within the niche that leaves them more vulnerable to displacement from the niche and loss of stemness. This raises the question of how to assign stem cell identity and stem cell number.

If stem cells are defined by their long-term tissue maintaining *potential* in steady-state, one would have to say that *all* 14-16 central and border $Lgr5^+$ CBC cells retain this capacity. However, as we have seen, not all stem cells are, at any given time, equally potent. Formally, we can dissect this variability by questioning the probability that a marked stem cell will eventually colonize a crypt. In the original variant of the neutral drift dynamics model, where all 16 cells were considered equipotent at any instant, this probability was simply 1 in 16 – any stem cell could clonally colonize the crypt. In the present case, this probability will vary according to whether stem cells are positioned in the border or central region. From a numerical simulation of the model dynamics, with parameters defined as

above, we find that an individual central stem cell has a long-term survival probability of around 0.094, while for a border stem cell it is 0.031, a factor 3 smaller. Of course, the precise values will depend on the actual number of functional Lgr5$^+$ cells, which we have taken as 8 in both layers. (Indeed, one may consider a more refined partitioning of the crypt base region into multiple layers, leading to a gradient or continuum of long-term survival potential.) If instead we use long-term survival as a defining feature of a stem cell then, by the nature of the drift dynamics, for any given crypt, there is only ever one stem cell at any given time that will persist long-term.

Finally, through rapid convergence of the clone dynamics onto one-dimensional neutral drift behaviour, one can use the model parameter, N_{stem}, to define effective stem cell number. However, it would be hasty to conclude that this figure translates to a defined or functionally distinct population of cells.

Challenging the model of stem cell heterogeneity

Although the ability of the new 'two-compartment' model to recapitulate the intermediate and long-term clone fate behaviour provides circumstantial evidence that cells that transfer from the border to central region function as stem cells, with long-term self-renewal potential, for the relatively short-times for which *in vivo* live-imaging is viable, it is not possible to confirm this directly. Therefore, it is useful to find further methods to challenge the integrity of the model. To this end, in the main text, we reported on the results of a regeneration assay following the targeted ablation of Lgr5 expressing cells using diphtheria toxin.

In the course of regeneration Lgr5$^-$ cells, recently detached from the niche, are able to transit back to the niche region and reacquire Lgr5 expression and stem cell identity. From the form of the clonal fate data, it is apparent that this transition occurs sporadically and at a relatively low frequency. In particular, at 3 days post-ablation, crypts show a wide range of variability in the degree of recovery, with some stem cell compartments almost fully replenished while, in others, recovery is sparse with only small clusters of Lgr5$^+$ cells at the crypt base. The cohesion of these clusters (Supplementary Video 6) suggests that their origin lies in the clonal expansion of individual TA cells that have transferred to the niche border region and upregulated expression of Lgr5.

To challenge the two-compartment model, we questioned what would be the predicted distribution of Lgr5 expressing cells between the border and central niche region, disaggregated by total cluster size, if we assumed that clonal expansion follows from the sporadic 'appearance' of an

Lgr5^{+} stem cell in the border region. Once a cell in the border region has regained Lgr5 expression and recovered stem cell potential, we assumed that its further clonal evolution would be defined by the same dynamics as steady-state. However, in this case, stem cell division may be uncompensated by displacement and loss of a neighbouring cell when there is no neighbour to displace. Such dynamics leads to the rapid repopulation of the stem cell niche compartment and the gradual restoration of homeostasis. To address quantitatively the resulting regeneration process, we again made use of a stochastic simulation to monitor the clonal dynamics imposing the same rate constants for the individual processes (cf. Fig. 3a).

Since crypt recovery is variable, reflecting the sporadic nature of the reacquisition of stem cell potential, and *in vivo* live-imaging of the regeneration process is currently unfeasible (retracing of non-fluorescent crypts was not feasible), it was not possible to resolve time course data. We therefore chose to characterise the recovery process by measuring the relative numbers of border and central cells in clusters of different sizes taking the chase period to be around 3 days. Significantly, we found that the relative border/central cell ratio for a given clone size was not very sensitive to the particular choice of the chase time, and the figure of 3 days was consistent with the experimental protocol. When compared to the data, we found that the model provided a remarkably good prediction of the cluster data, capturing the bias of small clusters towards the occupancy of the border compartment, and the restoration of parity between the border and central regions in larger clusters (Fig. 4c).

Supplementary references:

31 Wright, N. A. & Alison, M. *The biology of epithelial cell populations: volume 2*. Vol. 2 708 (Oxford University Press, USA, 1985).

Supplementary Video Legends

Supplementary Video 1 | 3D reconstruction of a crypt containing $Lgr5^+$ CBC cells. $Lgr5^+$ CBC cells are shown in green, non-CBC cells are shown in red and Collagen 1 is shown in blue.

Supplementary Video 2 | Dynamics of $Lgr5^+$ CBC cells at the crypt base. Left, time series of $Lgr5^+$ CBC cells in a crypt. In the cartoons, $Lgr5^+$ CBC cells are highlighted and the moving cells are colour coded. In the right two videos cell tracks are shown by lines and the centre of cells is indicated by dots. The time is indicated in hours. Scale bar, 20 μm.

Supplementary Video 3 | Dividing and moving Lgr5+ CBC cells. Three days after Tamoxifen administration, mice were imaged in real-time. $Lgr5^+$ CBC cells within the entire stem cell compartment of a single crypt are projected onto one plane and are shown over time. Note the outlined cells that divide and move.

Supplementary Video 4 | $Lgr5^+$ CBC cells get expelled from the stem cell niche. Three days after Tamoxifen administration, mice were imaged in real-time. $Lgr5^+$ CBC cells within the entire stem cell compartment of a single crypt are projected onto one plane and they are shown over time. Note the outlined cells that divide, move, and disappear.

Supplementary Video 5 | The heterogeneous recovery of stem cells following targeted ablation. In mice where the human DT receptor (DTR) fused to EGFP was knocked in the Lgr5 locus ($Lgr5^{DTR:EGFP}$), $Lgr5^+$ cells were fully ablated using diphtheria toxin (DT) injection. The recovery was monitored by acquiring a Z-stack at 58hrs. The video shows the Z-stack. The empty crypts are indicated with yellow. The crypts containing one recovered cell are indicated with red. The crypts containing more than 1 recovered cell are indicated with grey.

Supplementary Video 6 | The cohesion of clusters of $Lgr5^+$ cells. In mice where the human DT receptor (DTR) fused to EGFP was knocked in the Lgr5 locus ($Lgr5^{DTR:EGFP}$), $Lgr5^+$ cells were fully ablated using diphtheria toxin (DT) injection. The video shows a 3D reconstruction of clusters of recovered $Lgr5^+$ cells from different sizes found 72hrs after ablation. Note the cohesion of these clusters, which is suggesting their origin lies in clonal expansion of cells.