

EXTENDED EXPERIMENTAL PROCEDURES

Analytical Solution to the Optimal Control Model

In this section we formulate our optimal control model for crypt development and present its analytical solution. Our model considers two cell populations that differ in their proliferative dynamics – $n(t)$ stem cells and $N(t)$ nonstem cells (Figure 2). Stem cells divide with rate β_n and remain within the crypt indefinitely. Nonstem cells divide with rate β_N and are extruded from the crypt at rate α . Stem cells can divide either symmetrically into two stem cells with probability $p(t)$ or asymmetrically into one stem cell and one nonstem cell with probability $1-p(t)$. The optimization goal is to transfer the initial state (n_0, N_0) to a final crypt size of (n_T, N_T) in the minimal time. We assume $n_T > n_0$, $N_T > N_0 = 0$, assumptions that are based on our experimental measurements (Figure 6).

The dynamics of the crypt populations is described by the state equations:

$$\frac{dn(t')}{dt'} = p(t')\beta_n n(t') \quad (1)$$

$$\frac{dN(t')}{dt'} = (1 - p(t'))\beta_n n(t') + (\beta_N - \alpha)N(t') \quad (2)$$

where t' denotes time in non-scaled units and where $0 \leq p(t') \leq 1$. By using the rescaled time $t = \beta_n t'$ the equations become:

$$\frac{dn(t)}{dt} = p(t)n(t) \quad (1)$$

$$\frac{dN(t)}{dt} = (1 - p(t))n(t) + bN(t) \quad (2)$$

where $b = (\beta_N - \alpha)/\beta_n$

The optimization goal is to minimize the time, T to obtain a mature crypt:

$$T = \int_0^T 1 dt \quad (3)$$

Subject to the constraints in Equations (1, 2), $0 \leq p(t) \leq 1$ and with the boundary conditions: $n(0) = n_0$, $N(0) = 0$, $n(T) = n_T > n_0$, $N(T) = N_T > 0$. Following Kirk (Kirk, 2004) we introduce Lagrange multipliers λ_1 , λ_2 for constraints (1) and (2) to form the Hamiltonian:

$$H(t) = 1 + \lambda_1 p n + \lambda_2 (1 - p)n + \lambda_2 b N = (\lambda_1 - \lambda_2) n p + 1 + \lambda_2 (n + b N) \quad (4)$$

Pontryagin's minimum theory states that a necessary condition for a control function $p(t)$ to minimize the total time T is that it minimizes the Hamiltonian of Equation (4) at each time point. Additionally, since the final time T is free and the Hamiltonian does not explicitly depend on time, the Hamiltonian is identically zero at the extremal trajectory (Kirk, 2004):

$$H(t) = 0 \quad (5)$$

Equation (4) indicates that the Hamiltonian is a linear function of $p(t)$ at any time point t . It will thus be minimized by setting p at either its minimal or maximal allowed value, depending on whether the slope in Equation (4), $(\lambda_1 - \lambda_2)n$ is positive or negative respectively. This solution is termed a 'bang-bang' control (Kirk, 2004; Macevicz and Oster, 1976; Perelson et al., 1976). Since $n(t) > 0$, the solution will depend on the sign of the switching function $\sigma(t) = \lambda_1(t) - \lambda_2(t)$:

$$p(t) = \begin{cases} 0 & \lambda_1(t) - \lambda_2(t) > 0 \\ 1 & \lambda_1(t) - \lambda_2(t) < 0 \end{cases} \quad (6)$$

This solution indicates that at any given time all stem cells should employ the same proliferation mode – either all stem cells divide symmetrically ($p(t) = 1$) or all stem cells divide asymmetrically ($p(t) = 0$). We will next show that the control function switches only once during the process, and will determine the switching time, as well as the total time T for the optimal solution. The Lagrange multipliers obey the following co-state equations (Kirk, 2004):

$$\frac{d\lambda_1(t)}{dt} = -\frac{\delta H}{\delta n} = -\lambda_2 + (\lambda_2 - \lambda_1)p \quad (7)$$

$$\frac{d\lambda_2(t)}{dt} = -\frac{\delta H}{dN} = -b\lambda_2 \quad (8)$$

Equation (8) does not depend on $p(t)$ and its solution is:

$$\lambda_2(t) = \lambda_2(T)e^{-b(t-T)} \quad (9)$$

To solve $\lambda_1(t)$ We will next consider the two possible cases at T , $p(T) = 1$ and $p(T) = 0$:

Case 1

$p(T) = 1$ (and $\lambda_1(T) < \lambda_2(T)$ from Equation (6)). From Equations (4, 5):

$$H(T) = 1 + \lambda_1(T)n_T + \lambda_2(T)bN_T = 0 \quad (10)$$

And thus:

$$\lambda_1(T) = -\frac{1 + \lambda_2(T)bN_T}{n_T} < \lambda_2(T) \quad (11)$$

We next set $p(T) = 1$ in Equation (7) to solve for $\lambda_1(t)$ at times approaching T :

$$\lambda_1(t) = \lambda_1(T)e^{-(t-T)} \quad (12)$$

Since $p(T) = 1$, the solution $p(t)$ will only be possible if there is a switch, that is $0 < \tau < T$ for which $\lambda_1(\tau) = \lambda_2(\tau)$. This is because $N(0) = 0$ and Equation (2) shows that $N(t)$ can only increase if $p(t) < 1$ for a part of the process. From Equations (9, 11, 12) this will only be possible if $\lambda_2(T) \leq 0$. Additionally, since $\lambda_1(T) < \lambda_2(T)$ we must have $b > 1$, or else $\lambda_1(t) < \lambda_2(t)$ and $p(t) = 1$ for all $0 < t < T$. Thus a reachable extremal solution for which $p(T) = 1$ is possible only if $b > 1$. We will next calculate the switching time and show that there is only one switch. At the transition point:

$$\lambda_1(\tau) = \lambda_1(T)e^{-(\tau-T)} = \lambda_2(T)e^{-b(\tau-T)} = \lambda_2(\tau) \quad (13)$$

The solution of Equation (13) is:

$$\tau = T + \frac{1}{b-1} \ln\left(\frac{\lambda_2(T)}{\lambda_1(T)}\right) = T + \frac{1}{b-1} \ln\left(-\frac{n_T \lambda_2(T)}{1 + bN_T \lambda_2(T)}\right) \quad (14)$$

where we have used Equation (11). Prior to the switch, at $t < \tau$, $p(t) = 0$. Using Equation (7) we find:

$$\lambda_1(t) = \lambda_1(\tau) + \frac{\lambda_2(T)}{b} [e^{-b(t-T)} - e^{-b(\tau-T)}] \quad (15)$$

Equation (15) indicates that $\lambda_1(t) > \lambda_2(t)$ for all $0 < t < \tau$, and thus the switch at τ is the only transition during the process. This solution can be intuitively understood by noting that if $b > 1$, $\beta_N - \alpha > \beta_n$, and the nonstem cell yield per nonstem cell is higher than that for a stem cell. It is thus more productive to generate as many nonstem cells first and then switch to producing stem cells, as existing nonstem cells would propagate their numbers faster than the flux obtained from existing stem cells. To summarize, the solution for which $p(T) = 1$ is:

$$p = \begin{cases} 0 & 0 < t < \tau \\ 1 & \tau < t < T \end{cases} \quad (16)$$

And requires $b > 1$.

We will next use Equations (1, 2, 16) to solve for the switching time τ and the final time T in terms of (n_0, N_0, n_T, N_T) . Solving Equation (1) for $t > \tau$:

$$n(t) = n_0 e^{t-\tau} \quad (17)$$

And using $n(T) = n_T$:

$$T - \tau = \ln\left(\frac{n_T}{n_0}\right) \quad (18)$$

Solving Equation (2) for $0 < t < \tau$, and using $N(0) = 0$:

$$N(t) = \frac{n_0}{b} (e^{bt} - 1) \quad (19)$$

And at $t > \tau$:

$$N(t) = N(\tau)e^{b(t-\tau)} = \frac{n_0}{b} (e^{b\tau} - 1)e^{b(t-\tau)} \quad (20)$$

Setting $N(T) = N_T$ in Equation (20) and using Equation (18):

$$\tau = \frac{1}{b} \ln \left(1 + \frac{bN_T}{n_0} \left(\frac{n_0}{n_T} \right)^b \right) \quad (21)$$

Finally using Equation (18):

$$T = \ln \left(\frac{n_T}{n_0} \right) + \frac{1}{b} \ln \left(1 + \frac{bN_T}{n_0} \left(\frac{n_0}{n_T} \right)^b \right) \quad (22)$$

Equations (21, 22) provide us with the switching time and the total time.

Case 2

We now consider the second possibility consistent with the bang-bang control, $p(T) = 0$, or using (6), $\lambda_1(T) > \lambda_2(T)$. From Equations (4, 5):

$$\lambda_2(T) = -\frac{1}{n_T + bN_T} \quad (23)$$

Equation (9) still describes the temporal dynamics of $\lambda_2(t)$ for $0 < t < T$, as this is independent of $p(t)$. To solve the dynamics of $\lambda_1(t)$ at times approaching T we set $p(t) = 0$ in Equation (7):

$$\lambda_1(t) = \lambda_1(T) - \lambda_2(T) \frac{1 - e^{-b(t-T)}}{b} = \lambda_1(T) + \frac{1 - e^{-b(t-T)}}{b(n_T + bN_T)} \quad (24)$$

And the switching function is:

$$\sigma(t) = \lambda_1(t) - \lambda_2(t) = \lambda_1(T) - \lambda_2(T) \left(\frac{1}{b} + \left(1 - \frac{1}{b} \right) e^{-b(t-T)} \right) \quad (25)$$

Since $\sigma(T) > 0$ (from Equation (6)) the solution will be feasible only if a switch $\sigma(\tau) = 0$ occurs at some time $0 < \tau < T$. This is because $n_T > n_0$ and an increase in stem cell number cannot occur if $p(t) = 0$ for all $0 < t < T$. Thus from Equation (25) $\sigma(t)$ will cross zero only if $b < 1$. By setting $\sigma(\tau) = 0$ we find the switching time:

$$\tau = T - \frac{1}{b} \log \left(\frac{b}{b-1} \right) - \frac{1}{b} \log \left(\frac{\lambda_1(T)}{\lambda_2(T)} - \frac{1}{b} \right) \quad (26)$$

At times prior to the switch ($0 < t < \tau$) we use $p(t) = 1$ in Equation (7) to solve for the switching function:

$$\sigma(t) = \lambda_1(\tau)e^{t-\tau} - \lambda_2(\tau)e^{b(t-\tau)} < 0 \quad (27)$$

Where we used $\lambda_1(\tau) = \lambda_2(\tau)$ and the fact that $b < 1$. Thus a switch occurs only once. To summarize case 2, if $b < 1$ the optimal solution is:

$$p = \begin{cases} 1 & 0 < t < \tau \\ 0 & \tau < t < T \end{cases} \quad (28)$$

Solving Equation (1) using Equation (28):

$$n(t) = n_0 e^t \quad (29)$$

And using $n(\tau) = n_T$:

$$\tau = \ln \left(\frac{n_T}{n_0} \right) \quad (30)$$

Solving Equation (2) for $\tau < t < T$ and using $N(\tau) = 0$:

$$N(t) = \frac{n_T}{b} (e^{b(t-\tau)} - 1) \quad (31)$$

And using $N(T) = N_T$:

$$T = \ln\left(\frac{n_T}{n_0}\right) + \frac{1}{b} \ln\left(1 + \frac{bN_T}{n_T}\right) \quad (32)$$

Note that Equations (32) and (22) converge as b approaches 1.

Singular Intervals

Equation (6) indicates that the control function (stem cell symmetric division probability, $p(t)$) will always assume either its upper or lower limit, depending on whether the switching function $\sigma(t) = \lambda_1(t) - \lambda_2(t)$ is negative or positive respectively. The control function switches between its limit values at times when the switching function is zero. Singular intervals occur if $\sigma(t) = 0$ over a non-zero time interval. For this to occur, all the temporal derivatives of $\sigma(t)$ should be identically zero over this interval. Thus in a singular interval we would have:

$$\sigma(t) = \lambda_1(t) - \lambda_2(t) = 0 \Rightarrow \lambda_1(t) = \lambda_2(t) \quad (33)$$

$$\frac{d\sigma(t)}{dt} = \frac{d\lambda_1(t)}{dt} - \frac{d\lambda_2(t)}{dt} = -\lambda_2 + (\lambda_2 - \lambda_1)p + b\lambda_2 = 0 \Rightarrow b = 1 \quad (34)$$

Where we used the co-state Equations (7, 8) in Equation (34). Thus in the specific case when $b = 1$ the control function cannot be determined over the entire developmental process. Indeed, this case implies that the nonstem cell yield per dividing nonstem cell ($\beta_N - \alpha$) is equal to that from a dividing stem cell (β_n), and thus all feasible stem cell division strategies (functions $p(t)$) that are able to transfer the system from the initial state (n_0, N_0) to the final state (n_T, N_T) will take the same time.

To summarize, the optimal solution for achieving a mature crypt in minimal time is:

$$\begin{aligned} &\beta_n < \beta_N - \alpha \\ &0 \leq t \leq \tau \quad p(t) = 0 \quad n(t) = n_0 \quad N(t) = \frac{n_0}{b} (e^{bt} - 1) \\ &\tau \leq t \leq T \quad p(t) = 1 \quad n(t) = n_0 e^{t-\tau} \quad N(t) = \frac{n_0}{b} (e^{b\tau} - 1) e^{b(t-\tau)} \\ &\beta_n > \beta_N - \alpha \\ &0 \leq t \leq \tau \quad p(t) = 1 \quad n(t) = n_0 e^t \quad N(t) = 0 \\ &\tau \leq t \leq T \quad p(t) = 0 \quad n(t) = n_T \quad N(t) = \frac{n_T}{b} (e^{b(t-\tau)} - 1) \end{aligned}$$

Bang-Bang Control Remains the Optimal Solution When Considering Fixed Life Span of Nonstem Cells

Our optimization model assumed that the rate at which a nonstem cell is extruded from the crypt is uniform and independent of its 'birth' time - the time when it first divided from a stem cell. Intestinal cells migrate along the crypt axis as they divide, and a more realistic representation could consider extrusion rates that depend on the number of divisions that nonstem cell have undergone since their birth. Thus rather than having an exponentially decaying life time with rate α the life span of a nonstem cell would be a step function - cells will divide for precisely u generations and at generation u will be extruded. To study this possibility we performed lineage simulations of this process and numerically searched for the optimal stem cell probability function using a combinatorial optimization algorithm.

For simplicity we considered synchronous divisions of all cells with equal rates for stem cells and nonstem cells ($\beta_n = \beta_N$). For each probability function $p(t)$, where $t = 1, 2, \dots$ are discrete generation times, we simulated 100 different trees in which non stem cells divided for exactly u generations before being extruded. All trees were initiated with one stem cell and required to reach a final preset number of stem cells and nonstem cells. We scored each resulting lineage tree by the minimal time required to obtain the final state (to accelerate the simulations we allowed final nonstem cell counts that were within the range of 5 cells from the required final number). Trees in which the required size was not attainable received a penalty score that is 2 generations higher than the minimal possible time. Each probability function was assigned an average score S_i in 100 iterations.

At each stage i we performed a random change in the probability function $p_i(t)$ and retested a new function $p_j(t)$. $p_j(t)$ was obtained by always changing the symmetric division probability at random at one randomly chosen time point, changing at random at a second time point with probability 50% and at a third time point with probability 10% (these rules were heuristically chosen to accelerate the search of the space of possible probability functions). The scoring function for the new probability, S_j , was recalculated and a simulated annealing decision rule was employed - the new function $p_j(t)$ was accepted either if $S_j < S_i$ or at random with probability $e^{-\Delta S/\eta}$, where $\Delta S = S_j - S_i$ (note that we favor lower scores or average times to a mature crypt). η serves as a 'temperature' which allows the search to avoid being stuck in local minima. Our simulations started with temperature $T = 0.5$ and lowered it by 4% every 100 steps

(Monte Carlo sweeps). Each simulation was initiated several times with different initial probability functions and was run until convergence. The search converged on the 'bang-bang' control each time (Figure S1).

Bang-Bang Control Remains the Optimal Solution When Considering Variable Extrusion Rates

Our optimization model assumed a constant extrusion rate throughout the crypt developmental process. However, this rate could potentially vary due to both crypt growth and to the simultaneous expansion of the villi. For example, if nonstem cells are migrating at a constant speed throughout the process, the rate at which they exit the crypts would increase as crypts enlarge. To explore these effects we performed numerical simulations of the developmental process and searched for the optimal proliferation strategy under a wide range of variable extrusion rates.

As in the simulations of fixed extrusion rates we performed Monte Carlo simulations where for simplicity we considered synchronous divisions of all cells with equal rates for stem cells and nonstem cells ($\beta_n = \beta_N$). Different proliferative probability functions $p(t)$ were iteratively tested for the time to obtain a mature crypt and accepted according to the same simulated annealing rule used in the fixed-life-span simulations. In the present simulations, however, we eliminated cells with a temporally-varying extrusion rate $\alpha(t)$. We tested a wide range of $\alpha(t)$, including monotonously increasing or decreasing functions between $0.05\beta_n$ and $0.8\beta_n$, as well as randomly fluctuating extrusion rates with a coefficient of variation ranging from 0.1 to 0.6. The bang-bang control solution achieved the mature crypt at the minimal time for all variable extrusion rates tested.

SUPPLEMENTAL REFERENCES

Kirk, D. (2004). *Optimal Control Theory: An Introduction* (Dover).

Macevicz, S., and Oster, G. (1976). Modeling social insect populations II: Optimal reproductive strategies in annual eusocial insect colonies. *Behav. Ecol. Sociobiol.* 1, 265–282.

Perelson, A.S., Mirmirani, M., and Oster, G.F. (1976). Optimal strategies in immunology. I. B-cell differentiation and proliferation. *J. Math. Biol.* 3, 325–367.

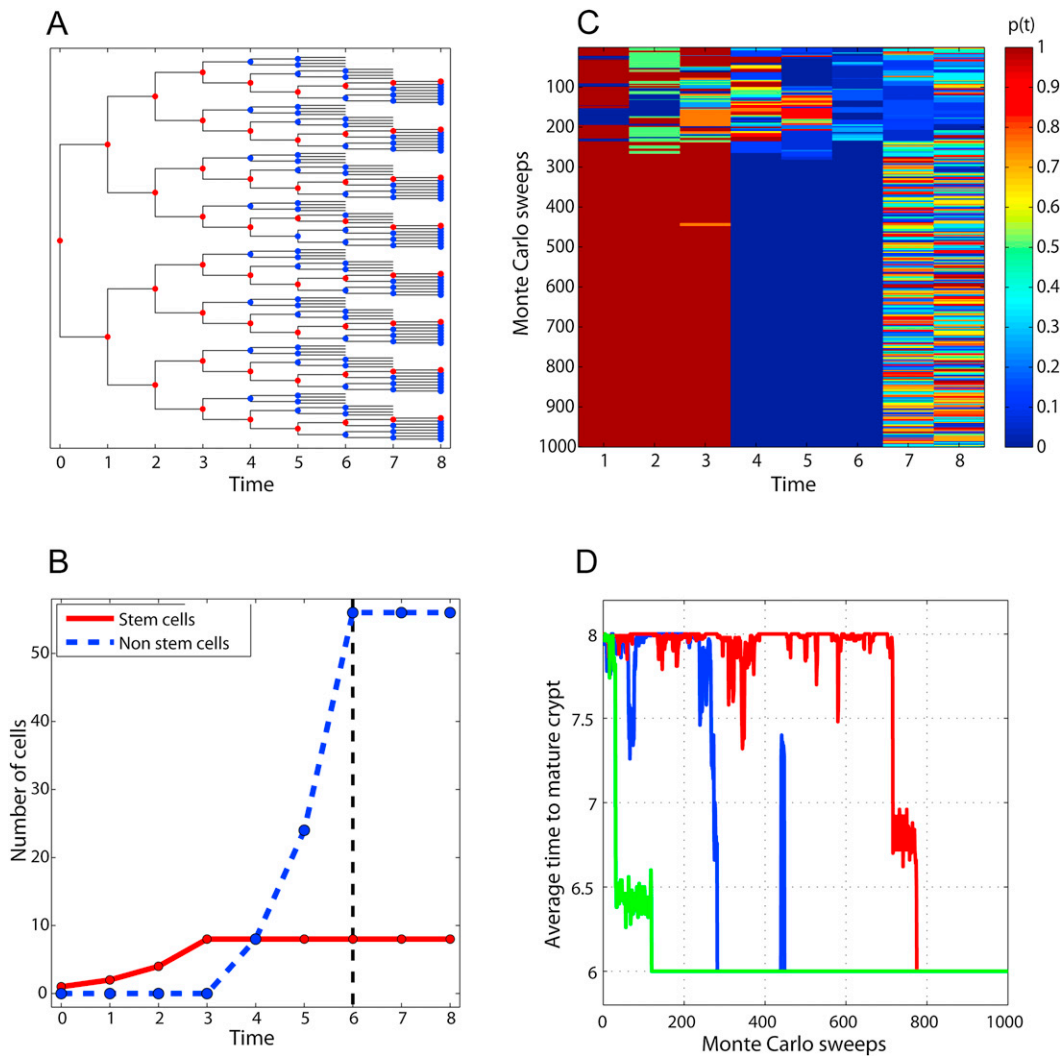


Figure S1. Bang-Bang Control of Symmetric Stem Cell Divisions Remains Optimal When Considering Fixed Life Span of Nonstem Cells, Related to Figure 2

(A) optimal lineage tree in which stem cells (red) first expand for three generations and then switch to asymmetric divisions generating nonstem cells (blue) for the remaining generations. Nonstem cells are extruded after two divisions.

(B) A required crypt size of 8 stem cells and 56 nonstem cells is achieved after 6 generations using the bang-bang control.

(C) Simulated annealing algorithm converges on the optimal bang-bang control solution. Each row shows a stem cell symmetric probability function at different Monte-Carlo sweeps, starting from a random probability function.

(D) Average time to achieve a mature crypt decreases toward the optimal solution as the simulated annealing algorithm proceeds. Shown are three representative iterations, where the blue represents the iteration in (C). All iterations converged on the bang-bang solution of (A and B).

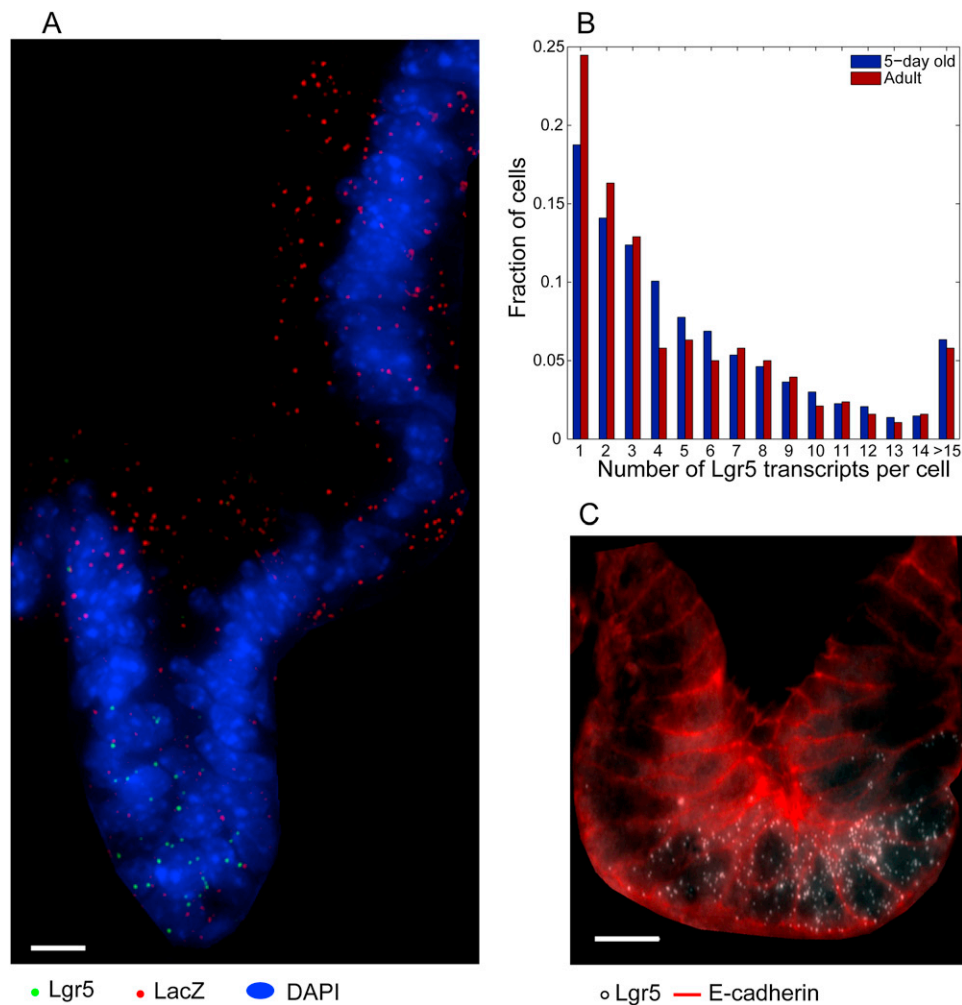


Figure S2. Related to Figure 4

(A) Lgr5 are the stem cells during crypt morphogenesis. Lgr5-Cre/Rosa26-lacZ 5-day old mice injected with tamoxifen and sacrificed 9 days later. Green dots are single Lgr5 transcripts, red dots are lacZ transcripts. Scale bar is 5 microns.

(B) Number of Lgr5 transcripts per cell in infant and adult mice. Quantification based on 6 optical sections spaced 0.3 microns apart.

(C) Single cells were segmented based on immunofluorescence with FITC-E-cadherin antibody which localizes at the cell borders (red). White dots are individual Lgr5 transcripts detected using TMR-labeled single-molecule FISH probes. Scale bar is 10 microns.

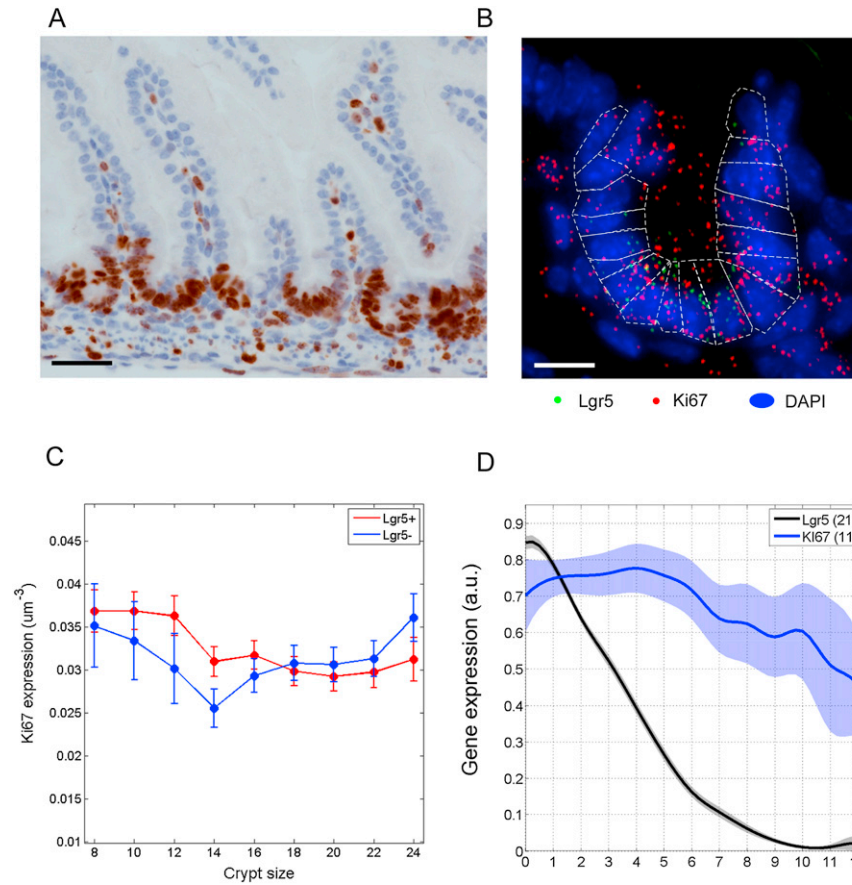


Figure S3. Related to Figure 5

(A) Intestinal crypts in 5-day old mice exhibit a uniform nuclear staining for Ki67 protein. Slide is counterstained with hematoxylin and eosin. Scale bar is 50 microns.

(B) Single-molecule fluorescence in situ co-hybridization for transcripts of Lgr5 (green dots) and the proliferation marker Ki67 (red dots). All dots represent single mRNA molecules. DAPI counterstaining is shown in blue. Segmented cells within the crypt are marked with dashed white lines. Segmentation was based on both DAPI counterstaining and simultaneous immunofluorescence with FITC-E-cadherin antibody which localizes at the cell borders. Scale bar is 10 microns.

(C) Ki67 transcript concentration is similar in Lgr5-positive and Lgr5-negative cells and remains constant along the developmental process. Shown are averages of Ki67 expression in single crypt cells versus the sizes of the crypts from which they were sampled along a moving window of ± 5 crypt sizes, errorbars are standard errors of the mean.

(D) Lgr5 stem cells are confined to crypt bottoms and are not extruded due to migration. Shown are the mean expression profiles of Lgr5 (black) and Ki67 (blue) at different positions along the crypt axis for duodenal crypts in 5-day old mice. Cell position 0 is the crypt base, profiles for each crypts were obtained by smoothing the profile of transcript concentrations with a moving window of 3 cell positions and dividing by its maximal value in the crypt. Patches are standard errors of the mean. The number of crypts used is shown in parentheses. Lgr5 cells are never found at villi and are confined to lower crypt positions, justifying our assumption that once formed they are not extruded from the crypts.

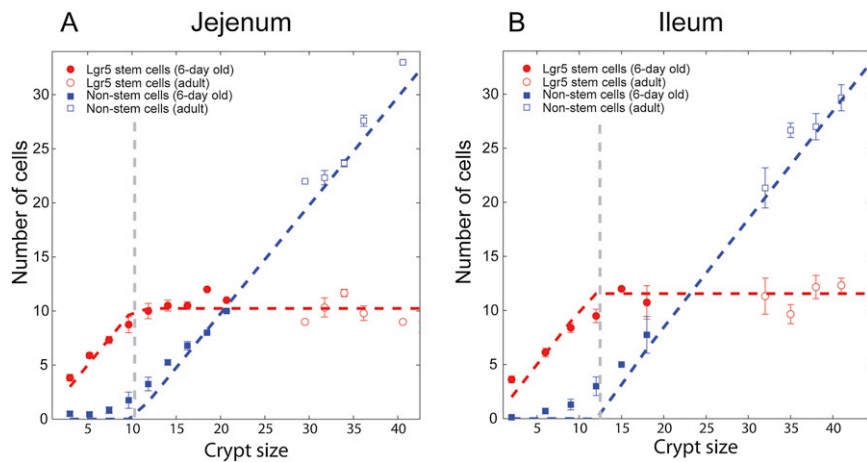


Figure S4. Related to Figure 6

Stem cells in developing crypts in the jejunum (A) and ileum (B) fit the bang-bang control dynamics. Small crypts are exclusively composed of Lgr5 stem cells. When the crypt reaches a size equal to the number of Lgr5 stem cells in the adult crypt (10 cells (in the jejunum) and 12 cells (in the ileum) per crypt longitudinal section, gray vertical dashed line) a transition to nonstem cell production occurs. Dashed red and blue curves are the dynamics predicted from the optimal control solution.