**Supplementary Materials and Methods.**

## Biophysical Modeling

To interpret the clonal fate data, we have made use of a quantitative biophysical modeling approach which parallels that used successfully in the analysis of other adult stem cell supported tissues. In the following section, we set out in more detail the experimental basis for this scheme, its adaptation to the current system, and we discuss its practical implementation to both the WT and mutant CySC behavior.

### Neutral drift dynamics of CySCs in WT tissue

As discussed in the main text, CySCs form a subpopulation of the Zfh1-expressing cyst cells, and are thought to retain direct contact with the somatic hub cells. These cells form a necklace of approximately N=13 cells around the hub and envelope the GSCs (Fig. 1A). As tissue turns over, CySCs give rise to progeny that detach from the hub and exit cell cycle.

Following heat-shock, one of the progeny of dividing CySCs may become genetically labeled. In homeostasis, precisely one half of these progeny become detached from the hub and differentiate as single labeled non-dividing cells. The other half, which belong to the CySC population, give rise to progeny which expand to occupy relatively contiguous domains of Zfh1-positive and Zfh1-negative cells (Figs. 1E,F and S2C,D). Significantly, these domains also expand around the hub while others become lost through commitment to differentiation. Together, these observations suggest that, as well as the potential for cell division leading to asymmetric fate outcome, CySCs also undergo progressive loss through commitment to differentiation and replacement, suggestive of population asymmetric self-renewal.

Similar behavior has been documented in several adult stem cell populations including the intestinal epithelium of both *Drosophila* posterior midgut (de Navascues et al., 2012) and mice (Lopez-Garcia et al., 2010). In particular, in mice, stem cells in both the small intestine and colon, which reside at or near the base of the crypt, undergo a similar process of population asymmetric self-renewal where the chance loss and replacement of stem cells from the niche region leads to neutral competition and a drift of surviving clones around the annulus of the crypt until the clone occupies the entire crypt base region (fixation) or it is lost altogether (extinction). Inspired by these earlier investigations, and the architectural similarities of the mammalian intestinal crypt and the *Drosophila* testis (discrete niche, limited number of stem cells, etc.), in the following we will make use of a simple biophysical modeling scheme that parallels the one used in gut and which captures the fundamental aspects of the clone dynamics in *Drosophila* testis. Crucially, our aim is to find the simplest model that captures the key elements of the dynamics. In particular, we do not seek to define a biophysical mechanistic basis for the regulation of CySC loss and replacement. While such a program would over-reach the validity of this approach, we note that any model of functionally equivalent (i.e. equipotent) CySCs, which has correlated loss and replacement of neighbors at its core, will converge onto the same clonal dynamics as that considered in the simplified scheme below.

In the following, we therefore consider a model of CySCs in WT tissue that form a single equipotent population in which any of these cells has an equal chance of being lost and replaced by its neighbor (Fig. 1A). As a simplification, we do not attempt to correlate the fate

behavior of CySCs with neighboring GSCs. The latter are simply regarded as a separate lineage with their own fate behavior. With this platform, CySCs form a one-dimensional chain of cells, which extends around the circumference of the hub. As CySCs proliferate, some become detached from the hub leading them to undergo commitment to differentiation and eventual loss. To maintain their overall number (homeostasis), CySC loss through detachment from the hub is perfectly compensated by the duplication of a neighboring CySC. By contrast, an asymmetrical CySC division leaves the CySC number unchanged.

In this one dimensional geometry, the resulting clone dynamics of the CySC compartment can be resolved analytically and depends on just two parameters - the loss/replacement rate, $\lambda$, of CySCs from the hub, and the total number of CySCs contacting the hub, N. Note that, if asymmetric CySC division contributes significantly to the dynamics of tissue turnover, the particular rate of CySC loss/replacement may be much smaller than the CySC division rate. Then, according to this dynamics, the chance $P_n(t)$ of finding a clone with $0 \le n \le N$ at time $t$ post-induction of a single labeled CySC is defined by the discrete Master equation (Lopez-Garcia et al., 2010),

$$\begin{aligned} \dot{P}_n(t) &= \lambda \hat{\Delta}_0 P_n(t) - \lambda\left[\delta_{n,1} + \delta_{n,-1} - 2\delta_{n,0}\right]P_0(t) \\ &\quad - \lambda\left[\delta_{n,N+1} + \delta_{n,N-1} - 2\delta_{n,N}\right]P_N(t) + \delta_{n,1}\delta(t), \quad (1) \end{aligned}$$

where, defining the one-dimensional lattice translation operator, $\hat{E}_m = \exp[m\hat{k}]$ with $[\hat{k}, n] = 1$ , $\hat{\Delta}_0 = (\hat{E}_1 + \hat{E}_{-1} - 2)$ denotes the lattice Laplacian. The first term on the right-hand side of the equation describes the random walk of the boundaries of a labeled clone, the second term reflects the potential for clone "extinction" (in which all marked CySCs become lost), while the third term reflects the possibility of clone "fixation" (in which all CySCs in the testis become labeled). The final term imposes the initial condition of one labeled cell per testis.

Formally, Eq. (1) describes a discrete diffusion equation on the interval $1 \le n \le N-1$, with absorbing boundaries at n=0,N imposed by the second and third terms of the equation. Here we have taken the time between consecutive loss/replacement events as random and statistically uncorrelated; a Poisson-random process. Although such an assumption ignores, for example, the refractory period between consecutive cell divisions, such effects are not significant and can be safely neglected for present purposes.

Integrating the Master equation, one may then show that the fraction of clones with $1 \le n \le N-1$ CySCs is given by (Lopez-Garcia et al., 2010)

$$P_n(t) = \frac{2}{N}\sum_{k=1}^{N-1} \sin\left[\frac{\pi k}{N}\right] \sin\left[\frac{\pi k n}{N}\right] \exp\left[-4\sin^2\left(\frac{\pi k}{2N}\right)\lambda t\right], \quad 1 \le n \le N-1,$$

while the fraction of clones that have either become extinct, $P_0(t)$, or have saturated the hub region, $P_N(t)$, is given respectively by,

$$P_0(t) = \frac{2}{N}\sum_{k=1}^{N-1}\cos^2\left[\frac{\pi k}{2N}\right]\left(1-\exp\left[-4\sin^2\left(\frac{\pi k}{2N}\right)\lambda t\right]\right),$$
$$P_N(t) = \frac{2}{N}\sum_{k=1}^{N-1}(-1)^{k+1}\cos^2\left[\frac{\pi k}{2N}\right]\left(1-\exp\left[-4\sin^2\left(\frac{\pi k}{2N}\right)\lambda t\right]\right).$$

If we consider only surviving clones, i.e. clones that retain at least one CySC, it is useful to define the "surviving" clone size distribution,

$$P_n^{surv.}(t) = \frac{P_n(t)}{1-P_0(t)}, \quad 1 \le n \le N.$$

At time scales in excess of the typical CySC loss time $1/\lambda$, but shorter than the time scale for the testis to drift to monoclonality, $N^2/\lambda$, these equations enter a scaling regime where (Lopez-Garcia et al., 2010)

$$P_n^{surv.}(t) \approx \frac{1}{<n(t)>} f\left(n/<n(t)>\right),$$

with $<n(t)> = \sqrt{\pi\lambda t}$ and $f(x) = \frac{\pi x}{2}\exp[-\pi x^2/4]$. In this regime, the chance of finding a clone with a size that is some given multiple of the average remains constant over time.

Although this analysis provides a useful platform to consider the dynamics of clonal evolution, its application to the present system is complicated by two factors. First, in the absence of a tightly-defined molecular marker for CySC, we have to find a surrogate to assess stem cell number within individual clones. Second, since the heat-shock leads to variable levels of induction, we have to consider the spread of GFP labeled cells in individual testes at the start of the chase. To address the first of these issues, we consider two experimental protocols, the quantitative analysis of which serves as a consistency check on each other. In the first strategy, we use MARCM clones that mis-express only membrane CD8-GFP to monitor proximity of cells to the hub as a proxy to identify the CySC population. As discussed in the text, this leads to a small but significant over-estimate in CySC number. In the second, we use Zfh1 expression of MARCM clones that mis-express only nuclear GFP, as a defined marker that encompasses, but extends well-beyond, the CySC population. In both cases, we then use the relative fraction of GFP labeled cells as a measure of the CySC content of the clone. For example, if 50% of cells in each domain (respectively proximate to niche or expressing Zfh1) are positive for GFP, we consider that 50% times N=13 = 7.5 CySCs are marked, etc. We note that a similar approach was used successfully by Lopez-Garcia et al. to estimate the number of effective stem cells in the intestinal crypt on the basis of the width of the migration stream of differentiated cells that reach the villus.

In relation to the induction frequency of CySCs, if we define the induction probability of an individual CySC following heat-shock as, q, the probability that a testis will host m labeled cells following induction is given by the binomial distribution,

$$Q_m = \frac{N!}{m!(N-m)!}q^m(1-q)^{N-m}.$$

If the induction frequency is low, qN<<1, we may make the approximation

$$Q_m \approx \frac{(qN)^m}{m!} \exp[-Nq].$$

In this form, the challenge of inducing CySCs at clonal density becomes evident: If we tune the incubation period of the heat-shock to ensure clonal density labeling, viz. qN<<1, the vast majority of testes will remain altogether unlabeled. Alternatively, if we induce at a frequency that ensures that the majority of testes contain at least one marked cell, then it is inevitable that some testes will become multiply induced. Although it is straightforward to extend the analysis of the Master equation above to an initial condition involving single clones with multiple labeled CySCs, the analysis of clonal evolution following the multiple induction of CySCs inside a single testis is technically more challenging. Instead, in this case, we can implement a numerical procedure involving a stochastic simulation to follow the clonal dynamics. It is this route that we follow.

As a starting point, we must first estimate the induction probability, q, following heat-shock. For this purpose, we can make use of the frequency of unlabeled testes to estimate the relative labeling efficiency. With some N=13 CySCs, the frequency of unlabeled testes at 2 dpci (~18%) translates to a labeling efficiency of around 1 in 10 (q=0.10±0.02) in the CD8-GFP MARCM experiment (see Figs. 1,2,7). As a consistency check, a fit of the predicted binomial distribution above with measurements of the distribution of discrete GFP-positive cell clusters at 2 dpci leads to a labeling efficiency of around 11% (Fig. 1H). Since marked differentiating Zfh1-positive cells stay within the domain, to fix the induction frequency using the second experimental protocol, it is more useful to use the long-term clone fixation probability. Since the clone fixation probability of each individual CySC, defined as the probability that its clonal progeny will eventually displace all other CySCs in a testis, is 1/N, the fraction of testes that remain labeled at long times is given by <m>/N, where

$$\langle m \rangle = \sum_{m=0}^{\infty} m Q_m = qN$$

denotes the average number of CySCs labeled at induction. With 16 testes with more than 50% labeled Zfh1+ cells out of a total of 89 testes examined at 28 days post-induction for the *FRT*$^{40A}$ WT flies (see Fig. S2), we infer a CySC induction probability of q=0.18±0.04, *i.e.* following heat-shock, some 10% of testes remain completely unlabeled, while the average labeling frequency is around qN is around 2 CySCs per testis. Similarly, for *FRT*$^{42D}$ in this second protocol, we infer a CySC induction probability of q=0.3±0.1

With the induction frequencies defined, we use a numerical stochastic simulation to follow the predicted evolution of GFP-labeled CySCs over time. By adjusting the CySC loss/replacement rate, we find that the model can provide a good agreement with the measured mean clone fraction of persisting clones (i.e. clones that retain at least one GFP-positve cell in the requisite domain) with λ=0.84±0.05 per day (Fig. 1G) for the first protocol (proximity to hub) and λ=1.0±0.1 per day for the second protocol (Zfh1-expression) (Fig. S2E,F). (Note that, for the latter, our analysis is limited to the longer-term data – 7 dpci and later. At shorter times, the labeled cell fraction of Zfh1-positive cells provides a relatively poor estimate of the CySC content: In short, the labeling protocol leads to the induction of CySCs and their immediate differentiated progeny (in equal proportion). Existing differentiated Zfh1-positive cells, which are post-mitotic, escape labeling. It is assumed that

each CySC contributes to the total Zfh1-positive pool, but this will not be reflected immediately after induction because the labeled cell has not had enough time to produce offspring. Therefore, by characterizing the clone size by the ratio of marked Zfh1-positive cells to the total, the short-term data necessarily underestimates the actual fraction of labeled CySCs. By contrast, at longer times, when the majority of differentiated Zfh1-positive cell progeny have become labeled, the estimate of clone size will become increasingly reliable.) Significantly, as well as recapitulating the mean clone size dependence, the model also accurately predicts the full cumulative clone size distribution over time for both experimental protocols (Figs. 1I, S2I,J), with departures attributable to statistical noise.

Finally, before turning to the *ptc* mutant data, it is worth noting that the time-dependence of the mean fractional clone size following multiple-cell induction converges rapidly onto that predicted analytically following the induction of single CySCs after a small time shift (5.5 days for *FRT*$^{42D}$ nls GFP MARCM clones or 3 days for all other experiments) of the induction time (Figs. 1G, S2E,F, orange lines). This coincidence follows from the rapid extinction and merger of marked CySCs, which allows the long-term dynamics to be dominated by single continuous domains of marked cells.

**Biased drift following *ptc* mutation**

With the WT behavior in hand, we now turn to consider the clonal evolution of the *ptc* mutant. In this case, it is evident from the comparison of the mean fractional clone size (Figs. 2C, S4C) with the WT control (Figs. 1G, S2F) in both experimental protocols, that *ptc* mutation results in an accelerated progression towards fixation. To assess whether the dynamics of the mutant clones in the WT background can be described as a biased drift process, we again turn to the one-dimensional modeling scheme. In this case, we suppose that, following the loss of a CySC through commitment to differentiation, a neighboring *ptc* mutant cell will have a higher chance of effecting its replacement through symmetrical cell division than a WT neighboring CySC. Indeed, as discussed in the main text, such an advantage can arise simply if the rate of CySC division is increased in the *ptc* mutant cell while the loss rate (*i.e.*, detachment from the hub) is unchanged from the WT value. In this case, as cells become detached, *ptc* mutant cells are able to effect their replacement more efficiently with a relative advantage that scales in proportion to the ratio of the cell division rates.

Once again, to define the clonal dynamics of *ptc* mutant CySCs in a field of WT CySCs, we will begin by considering a tissue with just a single marked CySC, turning later to consider the effect of multiple cell induction. As before, let us again define $P_n(t)$ as the probability of finding a *ptc* mutant clone with $0 \le n \le N$ CySCs at a time t post-labeling. Then, if we define $\lambda(1+\delta)$ as the loss/replacement rate leading to expansion of the mutant clone, and $\lambda(1-\delta)$ as the rate leading to contraction, we obtain the Master equation for the time evolution of the probability,

$$\begin{aligned}\dot{P}_n(t) = \lambda \hat{\Delta} P_n(t) &- \lambda\left[(1-\delta)\delta_{n,1} + (1+\delta)\delta_{n,-1} - 2\delta_{n,0}\right]P_0(t) \\ &- \lambda\left[(1-\delta)\delta_{n,N+1} + (1+\delta)\delta_{n,N-1} - 2\delta_{n,N}\right]P_N(t) + \delta_{n,1}\delta(t), \quad (2)\end{aligned}$$

where $\hat{\Delta} = \hat{\Delta}_0 - \delta(\hat{E}_1 - \hat{E}_{-1})$ denotes the lattice Laplacian with a bias. Once again, even in the presence of the bias, the Master equation can be solved analytically (Snippert et al., 2014). In particular, defining the parameters $v = \sqrt{(1+\delta)/(1-\delta)}$ and $\mu = \sqrt{(1-\delta^2)}$, one may show that

$$P_n(t) = \frac{2}{N} v^{n-1} \sum_{k=1}^{N-1} \sin\left[\frac{\pi k}{N}\right] \sin\left[\frac{\pi k n}{N}\right] \exp\left[-\mu f_k \lambda t\right], \quad 1 \leq n \leq N-1,$$

where $f_k = 2(1/\mu - 1) + 2\sin^2(\pi k / 2N)$, while

$$P_0(t) = \frac{2}{vN} \sum_{k=1}^{N-1} \frac{1}{f_k} \sin^2\left[\frac{\pi k}{2N}\right] \left(1 - \exp\left[-\mu f_k \lambda t\right]\right),$$

$$P_N(t) = \frac{2v^{N-1}}{N} \sum_{k=1}^{N-1} \frac{(-1)^{k+1}}{f_k} \sin^2\left[\frac{\pi k}{N}\right] \left(1 - \exp\left[-\mu f_k \lambda t\right]\right).$$

To follow the dynamics of the experimental system, once again we must consider the potential for multiple CySC induction. For the membrane CD8-GFP labeling system, using the frequency of unlabeled testes at 2 dpci, we find that the labeling efficiency of the *ptc* mutant is similar to the control at around q=0.1. For the nuclear-GFP labeling system, we cannot use the long-term clone recovery rate to fix the induction as we could in the control because the dynamics are now non-neutral. Instead, we suppose that clones are induced at the same frequency as that observed in the control (q=0.3, Fig. S2F,H), noting that, for the reasons outlined in the previous section, the long-term dynamics are relatively insensitive to the precise degree of labeling. Secondly, to further constrain the dynamics, we will assume that the loss/replacement rate of WT neighbors bordering a departing *ptc* mutant CySC, $\lambda(1-\delta)$ is equal to the loss/replacement rate obtained for the WT control. Then, adjusting the one remaining parameter, the degree of bias, we obtain a best fit of the model to the average clone size for a bias of $\delta=0.15\pm0.02$ for the protocol using the membrane CD8-GFP (Fig. 2C) and $\delta=0.12\pm0.04$ for the protocol using the nuclear-GFP (Fig. S4C). Significantly, with the same choice of parameters, the measured cumulative clone size distribution compares favorably with those predicted by the model over the range of time points (Fig. 2D, S4E). Once again, for the reasons outlined in the previous section, we note that the dynamics following mosaic labeling quickly converges onto that of single CySC-derived clones defined above following a time-shift of 5.5 days (Fig. S4C, orange line).

Finally, although the data is a little more noisy, we note that clonal dynamics of the *hpo* mutant can be accurately predicted by the same model parameters as that used for the *ptc* mutant (Fig. 7D,E).

**Fly Stocks and genotypes.**

All alleles used are described in Flybase (http://flybase.org). The double mutants $ex^{697}$, $FRT^{42D}$, *ptcS2* and $FRT^{42D}$, $ptc^{S2}$, *ykiB5* were obtained by recombination, selected for the FRT using food supplemented with Geneticin (Life Technologies) and screened for the presence of

the *ptc* mutation by lack of complementation with *ptc*$^{IIw}$. *yki*$^{B5}$ and *ex*$^{697}$ were scored first for presence of a *w* rescue then verified by lack of complementation with *yki*$^{R310X}$ or by presence of β-gal, respectively. The following genotypes were used in each figure :

Figure 1 :

B. *Oregon R*

C. *yw, hsflp*$^{122}$*, UAS-CD8-GFP/Y; ; Tub>Gal4, FRT*$^{82B}$*, Tub>Gal80/FRT*$^{82B}$

D-I. *yw, hsflp*$^{122}$*, UAS-CD8-GFP/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$; *Tub>Gal4/+*

Figure 2 :

A-E. *yw, hsflp*$^{122}$*, UAS-CD8-GFP/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$; *Tub>Gal4/+*

F. "control" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$

"*ptc*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$

Figure 3 :

A. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$

B-C. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$

D. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-Ci5Ncm5, UAS-Ci5m30/+*

E. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-Ptc RNAi/+*
(Ptc RNAi from NIG2411R1)

F-G. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-Hop/+*

H. "control" as Fig. 3B; "UAS-Hop" as Fig. 3F; "UAS-Ci$^{Act}$" as Fig. 3D; "UAS-*ptc* RNAi" as Fig. 3E.

I. “control” and “*ptc*” are as Fig. 2F

"control; *Stat92E*/+ " is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*; FRT*$^{82B}$*, Stat92E*$^{85c9}$*, e/+*

“*ptc; Stat92E/+*” is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; FRT*$^{82B}$*, Stat92E*$^{85c9}$*, e/+*

<u>Figure 4 :</u>

A-B. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$

C. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-βPS-Integrin/+*

D. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-DE-cadherin/+*

E. *yw, hsflp*$^{122}$*, UAS-CD8-GFP, Tub>Gal4/Y; ; Tub>Gal80, FRT*$^{80B}$*/FRT*$^{80B}$

F. *yw, hsflp*$^{122}$*, UAS-CD8-GFP, Tub>Gal4/Y; ; Tub>Gal80, FRT*$^{80B}$*/rhea*$^{1}$, *FRT*$^{80B}$

G. "control" as Fig. 4E and "*rhea*$^{1}$" as Fig. 4F

H. “control” is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$

"UAS-βPS integrin" is as Fig. 4C

"UAS-TalinH" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-TalinH-GFP/+*

"UAS-DE-Cad" is as Fig. 4D.

I. "control" and "*ptc*" are as Fig. 2F

"control; *rhea*$^{1}$/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*; FRT*$^{80B}$, *rhea*$^{1}$*/+*

"*ptc; α-cat/+*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; α-Cat*$^{1}$*/+*

"*ptc; rhea*$^{6\text{-}66}$*/+*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; rhea*$^{6\text{-}66}$*/+*,

"*ptc; rhea*$^{1}$*/+*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; FRT*$^{80B}$*, rhea*$^{1}$*/+*

<u>Figure 5 :</u>

A. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$

B. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$

C. *yw, hsflp*$^{122}$*/Y; FRT*$^{42D}$*, ptc*$^{S2}$*/FRT*$^{42D}$*, arm-lacZ; stg-GFP*$^{YD0246}$*/+*

D. *yw, hsflp*$^{122}$*/Y; FRT*$^{42D}$*, ptc*$^{S2}$*/FRT*$^{42D}$*, arm-lacZ; PCNA-GFP/+*

E. "control" and "*ptc*" as Fig. 5A and 5B

Figure 6 :

A. "control" is *yw, hsflp*$^{122}$*, UAS-CD8-GFP/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$; *Tub>Gal4/+*

"*ptc*" is *yw, hsflp*$^{122}$*, UAS-CD8-GFP/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$; *Tub>Gal4/+*

"*ptc; stg/+*" is *yw, hsflp*$^{122}$*, UAS-CD8-GFP/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$; *Tub>Gal4/stg*$^{4}$*, e*

B. "control" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$

"control; *stg*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$; *stg*$^{4}$*, e*/+

"*ptc*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$

"*ptc; stg/+*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; stg*$^{4}$*, e/+*

C. "control" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$

"control; *Akt*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$ *, Tub>Gal80/FRT*$^{42D}$*; Akt1*$^{04226}$*/+*

"control; *InR*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*; InR*$^{E19}$*/+*

"control; *S6k*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*; S6k*$^{L1}$*/+*

"control; *cdk2*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*; cdk2*$^{3}$*/+*

"control, *E2f*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*; E2f*$^{i2}$*, e/+*

"*ptc*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$

"*ptc*; *Akt/+*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$ *, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; Akt1*$^{04226}$*/+*

"*ptc*; *InR*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; InR*$^{E19}$*/+*

"*ptc*; *S6k*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; S6k*$^{L1}$*/+*

"*ptc*; *cdk2*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; cdk2*$^{3}$*/+*

"*ptc*, *E2f*/+" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; E2f*$^{i2}$*, e/+*

D. "control" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$ */FRT*$^{40A}$

“UAS-CycE, UAS-Stg” is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-CyclinE, UAS-String/+*

E. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-CyclinE, UAS-String/+*

F. as in Fig. 6D.

Figure 7:

A. yw, hsflp$^{122}$/Y; ex$^{697}$FRT$^{39}$/CyO; TM2/TM6B (i.e., ex-lacZ)

B. yw, hsflp$^{122}$, UAS-CD8-GFP/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$, hpo$^{KC202}$; Tub>Gal4/+

C-E. as in Fig. 7B.

F. "control" is yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$

"ptc" is yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$, ptc$^{S2}$

"hpo" is yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$, hpo$^{KC202}$

"hpo; stg/+" is yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$, hpo$^{KC202}$; stg$^{4}$, e/+

G. "control" is yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$ /FRT$^{40A}$

"UAS-Yki$^{Act}$" is yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$; UAS-yki$^{S111A,S168A,S250A:V5}$/+

H. yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$; UAS-yki$^{S111A,S168A,S250A:V5}$/+

Figure 8 :

A. yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$, yki$^{B5}$

B. yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$, ptc$^{S2}$

C. yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$, ptc$^{S2}$, yki$^{B5}$

D. yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/ex$^{697}$, FRT$^{42D}$, ptc$^{S2}$

E. "control" is yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$

"*ptc*" is *yw, hsflp*$^{122}$; *FRT*$^{42D}$, *ubi-GFP/FRT*$^{42D}$, *ptc*$^{S2}$

"*yki*" is *yw, hsflp*$^{122}$; *FRT*$^{42D}$, *ubi-GFP/FRT*$^{42D}$, *yki*$^{B5}$

"*ptc yki*" is *yw, hsflp*$^{122}$; *FRT*$^{42D}$, *ubi-GFP/FRT*$^{42D}$, *ptc*$^{S2}$, *yki*$^{B5}$

"*hpo*" is *yw, hsflp*$^{122}$; *FRT*$^{42D}$, *ubi-GFP/FRT*$^{42D}$, *hpo*$^{KC202}$

F. "control" and "*yki*" as Fig. 8E

Figure S1 :

A. *yw, hsflp*$^{122}$; *Sp/+; PCNA-GFP/+*

B. CantonS

Figure S2 :

A, C, E, G, I. *yw, hsflp*$^{122}$, *UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$/*FRT*$^{40A}$

B, D, F, H, J. *yw, hsflp*$^{122}$, *UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$, *Tub>Gal80/FRT*$^{42D}$

Figure S3 :

A. *yw, hsflp*$^{122}$, *UAS-CD8-GFP/Y; FRT*$^{42D}$, *Tub>Gal80/FRT*$^{42D}$; *Tub>Gal4/+*

B. *yw, hsflp*$^{122}$, *UAS-CD8-GFP/Y; FRT*$^{42D}$, *Tub>Gal80/FRT*$^{42D}$, *ptc*$^{S2}$; *Tub>Gal4/+*

C. *yw, hsflp*$^{122}$, *UAS-CD8-GFP/Y; FRT*$^{42D}$, *Tub>Gal80/FRT*$^{42D}$, *hpo*$^{KC202}$; *Tub>Gal4/+*

Figure S4 :

A.E. *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$, ptc$^{S2}$*

F. "control" is *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$*

"*ptc*" is *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$, ptc$^{S2}$*

Figure S5 :

A,B. *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$, ptc$^{S2}$*

Figure S6 :

A. *yw, hsflp$^{122}$; UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$; UAS-dMyc/+*

B. Top: “control” is *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$*

"UAS-dMyc" is *yw, hsflp$^{122}$; UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$; UAS-dMyc/+*

Bottom: "control" is *yw, hsflp$^{122}$; FRT$^{42D}$, ubi-GFP/FRT$^{42D}$*

“M$^{+}$” is *yw, hsflp$^{122}$; FRT$^{42D}$/FRT$^{42D}$, M(2)58F, ubi-GFP*

C. *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$*

D. *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$, ptc$^{S2}$*

E. . "control" is *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$*

"*ptc*" is *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; FRT$^{42D}$, Tub>Gal80/FRT$^{42D}$, ptc$^{S2}$*

"*ptc; hid/+*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$*; hid*$^{P05014}$*/+*

Figure S7 :

A,B. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$

C,D. *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$, *Pten*$^{dj189}$

E,F. *yw, hsflp*$^{122}$*, UAS-CD8-GFP/Y; ; Tub>Gal4, FRT*$^{82B}$*, Tub>Gal80/FRT*$^{82B}$

G, H. *yw,hsflp*$^{122}$*, UAS-CD8-GFP/Y; ; Tub>Gal4, FRT*$^{82B}$ *, Tub>Gal80/FRT*$^{82B}$, *Tsc1*$^{29}$

I. "FRT$^{40A}$ control" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$

"UAS-dp110" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-Dp110/+*

"*Pten*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$, *Pten*$^{dj189}$

"UAS-dMyc" is *yw, hsflp*$^{122}$*; UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT*$^{40A}$*/FRT*$^{40A}$*; UAS-dMyc/+*

"FRT$^{82B}$ control" is *yw, hsflp*$^{122}$*, UAS-CD8-GFP/Y; ; Tub>Gal4, FRT*$^{82B}$*, Tub>Gal80/FRT*$^{82B}$

"*Tsc1*" is *yw,hsflp*$^{122}$*, UAS-CD8-GFP/Y; ; Tub>Gal4, FRT*$^{82B}$ *, Tub>Gal80/FRT*$^{82B}$, *Tsc1*$^{29}$

Table S1:

"control" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$

"*ptc*" is *yw, hsflp*$^{122}$*, UAS-nlsGFP, Tub>Gal4/Y; FRT*$^{42D}$*, Tub>Gal80/FRT*$^{42D}$*, ptc*$^{S2}$

Table S2 :

"MARCM FRT$^{40A}$ control" is *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$*

"MARCM FRT$^{40A}$, UAS-Dp110" is *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$; UAS-Dp110/+*

"MARCM FRT$^{40A}$ *Pten$^{dj189}$*" is *yw, hsflp$^{122}$, UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$, Pten$^{dj189}$*

"MARCM FRT$^{40A}$ UAS-dMyc" is *yw, hsflp$^{122}$; UAS-nlsGFP, Tub>Gal4/Y; Tub>Gal80, FRT$^{40A}$/FRT$^{40A}$; UAS-dMyc/+*

"MARCM FRT$^{82B}$ control" is *yw, hsflp$^{122}$, UAS-CD8-GFP/Y; ; Tub>Gal4, FRT$^{82B}$, Tub>Gal80/FRT$^{82B}$*

"MARCM FRT$^{82B}$ *Tsc1$^{29}$* is *yw, hsflp$^{122}$, UAS-CD8-GFP/Y; ; Tub>Gal4, FRT$^{82B}$ Tub>Gal80/FRT$^{82B}$*, *Tsc1$^{29}$*

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