Supplementary Note A

Quantitative models of stem cell evolution

In this Supplementary Note we describe two Markov chains that can be used to explore the behavior of the population asymmetry models, B and C, described in Figure 1 of the main text.

Both models describe the evolution through time of a population of stem cells whose size is maintained at N cells. The stem cells are of two types, labeled normal and variant, the variant cells having a division rate that is c times that of the normal cells, for some value of $c > 0$. The Markov chains we study are denoted by $\{X(t), t \geq 0\}$, where $X(t)$ is the number of variant stem cells at time t. The state space of these chains is $\mathcal{S} = \{0, 1, \ldots, N\}$, states $\mathcal{A} = \{0, N\}$ are absorbing, 0 corresponding to loss of the variant type, and N to loss of the normal type.

The dynamics of Model B

In this model the variant cells divide at rate $c\tau$ and the normal cells at rate τ. On division, either type of cell produces 0, 1 or 2 offspring of the parental type, with probabilities q, w, p respectively, where $p + q + w = 1$. To maintain constant size, an individual is added or deleted as necessary, the type being chosen in *proportion to the overall birthrate of that type* (as opposed to its proportion in the population, as in Model C).

By considering the possible outcomes at a division, we find that the transition rates are

$$\begin{aligned} r \to r+1 \quad \text{at rate } \lambda_j := \; & rc\tau p \frac{(N-r)\tau}{(N-r)\tau + rc\tau} + (N-r)\tau q \frac{rc\tau}{(N-r)\tau + rc\tau} \\ = \; & \frac{r(N-r)\tau c(q+p)}{N-r+rc} \end{aligned} \tag{1}$$

and

$$\begin{aligned} r \to r-1 \quad \text{at rate } \mu_j := \; & rc\tau q \frac{(N-r)\tau}{(N-r)\tau + rc\tau} + (N-r)\tau p \frac{rc\tau}{(N-r)\tau + rc\tau} \\ = \; & \frac{r(N-r)\tau c(q+p)}{N-r+rc} \end{aligned} \tag{2}$$

To calculate the rates in (1) for example, there are two cases. We can have a division in one of the r variant cells (rate $rc\tau$), that cell produces two

variant offspring cells (chance p), and we remove a normal cell to keep the total numbers constant. The chance of this is

$$\frac{(N-r)\tau}{(N-r)\tau + rc\tau} = \frac{N-r}{N-r+rc}.$$

Alternatively, we can have a division in one of the $N-r$ normal cells (rate $(N-r)\tau$), the normal cell is replaced by 0 normal cells (chance q), and a variant cell is chosen to be added to make the population size up to N. The chance of this is

$$\frac{rc\tau}{(N-r)\tau + rc\tau} = \frac{rc}{N-r+rc}.$$

Combining these possibilities yields (1).

The dynamics of Model C

Each cell is either a variant (fast) stem cell or an ordinary stem cell. Replication works as follows: variant stem cells divide at rate $c\tau$ and produce two variant stem cell offspring, while normal stem cells divide at rate τ and produce normal stem cell offspring. To maintain a constant population size, a randomly chosen cell is removed.

The transition rates of Model B are given by

$$j \to \begin{cases} j+1 & \text{at rate } jc\tau\,(N-j)/N \\ j-1 & \text{at rate } (N-j)\tau\,j/N \end{cases} \tag{3}$$

Models B and C are examples of birth-and-death processes with absorbing boundaries; indeed, Model C is a Moran model (Moran, 1958). While some explicit formulae for quantities of interest here are known, it is simpler to take a more computational approach, particularly as we are focusing on a single value of $N = 16$. Note also that the parameter τ serves to calibrate the time scale. In these notes we take $\tau = 1$ for both models.

Computational approach

We denote the transition matrix of either chain by

$$P(t) := (p_{ij}(t), i, j \in \mathcal{S}) = \exp(Qt),$$

where $Q = (q_{ij},\ i, j \in \mathcal{S})$ is the rate matrix of the process; this satisfies

$$\sum_{j \in \mathcal{S}} q_{ij} = 0, \text{ for all } i \in \mathcal{S}.$$

We also make use of the expected time, m_{ij}, spent in state j, starting from state i, before loss or fixation of the variant type:

$$m_{ij} = \int_0^\infty p_{ij}(u)\, du, \quad i, j \in \mathcal{T}.$$

Writing

$$Q_0 = (q_{ij},\, i, j \in \mathcal{T}),$$

we recall (cf. Tavaré, 1979) that

$$M = (m_{ij}) = (-Q_0)^{-1}.$$

The probability l_i that the variant cell disappears from the population, starting from $X(0) = i$ initial copies, is given by the ith element of the column vector

$$l = Mq_0, \tag{4}$$

where $q = (q_{10}, \ldots, q_{N-1,0})'$.

The mean of the time T to loss of *either* cell type starting from i variant stem cells is given by

$$\mathbb{E}(T|X(0) = i) = \sum_{j=1}^{N-1} m_{ij}, \tag{5}$$

whereas the mean time to loss of the variant type, conditional on that type being lost, is given by

$$\mathbb{E}(T|X(0) = i, X(\infty) = 0) = \sum_{j=1}^{N-1} n_{ij}\, l_j / l_i, \tag{6}$$

where l_i is given in (4).

Next we discuss the "half-life of a variant stem cell". This term needs some definition. To this end, the distribution function of the time to disappearance of the variant type, conditional on this happening, is

$$\begin{aligned} G_i(t) &:= \mathbb{P}(T_0 \le t \mid T_0 < \infty) \\ &= \mathbb{P}(X(t) = 0 \mid X(0) = i, X(\infty) = 0) \\ &= p_{i0}(t)/l_i, \end{aligned} \tag{7}$$

For $\alpha \in (0,1)$, we define the percentiles of G_i by

$$t_{100\alpha} := \inf_{t \geq 0} \{G_i(t) - \alpha = 0\} \tag{8}$$

When $i = 1$, the value of t_{50} is called the half-life of a new variant.

We conclude this section by computing the average number of stem cells of variant lineage, when present. This quantity is interpreted as

$$\mathbb{E}(X(t)|X(t) > 0, X(0) = i),$$

The parameter i is the initial number of the variant type, and is normally 1. This is easy to compute, because it is

$$\frac{\sum_{j=1}^{n} j p_{ij}(t)}{1 - p_{i0}(t)}. \tag{9}$$

The quantity $1 - p_{i0}(t)$ is the probability that the variant type is present at time t.

The quantities in this section are readily computed using R, and suitable code is given below.

Numerical results for Model B

Loss probability and average time to loss of a variant cell

Table 1 gives the probability l_1 that a single variant cell will be lost from the population, and the average time such a loss takes. l_1 is independent of the parameters because $\lambda_j = \mu_j$ for all j.

c	0.1	0.2	0.5	0.8				
l_1	0.9375	0.9375	0.9375	0.9375				
$\mathbb{E}(T\vert \text{ hit } 0)$	41.79	21.39	9.158	6.099				
c	1.0	1.1	1.2	1.5	2.0	3.0	4.0	5.0
l_1	0.9375	0.9375	0.9375	0.9375	0.9375	0.9375	0.9375	0.9375
$\mathbb{E}(T\vert \text{ hit } 0)$	5.079	4.708	4.399	3.719	3.039	2.360	2.020	1.816

Table 1. Probability that variant cell type goes extinct and mean time to loss of the variant type, conditional on loss, starting from $X(0) = 1$, for various values of c.

Half-life of variant lineage, when present

The half-life t_{50} and the 90th percentile t_{90} defined in (8) are given in Table 2.

c	0.1	0.2	0.5	0.8				
t_{50}	20.16	10.16	4.159	2.620				
t_{90}	190.22	98.34	43.14	26.70				
c	1.0	1.1	1.2	1.5	2.0	3.0	4.0	5.0
t_{50}	1.900	1.599	1.271	0.736	0.379	0.160	0.091	0.059
t_{90}	13.00	8.259	5.485	2.263	0.941	0.345	0.185	0.117

Table 2. Half-life of variant cell type, conditional on loss, starting from a single variant cell, for various values of c.

Average number of variant stem cells when present

	time t			
c	4.5	6.0	7.5	9.0
0.1	0.8173	0.7704	0.7286	0.6911
0.2	0.6926	0.6284	0.5751	0.5301
0.5	0.4805	0.4104	0.3586	0.3187
0.8	0.3739	0.3114	0.2676	0.2353
1.0	0.3288	0.2716	0.2325	0.2041
1.1	0.3110	0.2562	0.2191	0.1923
1.2	0.2955	0.2431	0.2078	0.1825
1.5	0.2596	0.2131	0.1824	0.1607
2.0	0.2214	0.1823	0.1569	0.1392
3.0	0.1820	0.1517	0.1323	0.1188
4.0	0.1626	0.1371	0.1209	0.1095
5.0	0.1513	0.1288	0.1144	0.1043

Table 3. Probability $\mathbb{P}(X(t) > 0)$ that the variant type is still present, for various values of c and t, with $p = q = 0.25$, and $X(0) = 1$.

	time t			
c	4.5	6.0	7.5	9.0
0.1	1.233	1.298	1.372	1.447
0.2	1.444	1.591	1.739	1.886
0.5	2.081	2.436	2.789	3.137
0.8	2.675	3.211	3.736	4.249
1.0	3.041	3.682	4.302	4.901
1.1	3.216	3.903	4.564	5.199
1.2	3.384	4.114	4.812	5.480
1.5	3.852	4.692	5.481	6.223
2.0	4.517	5.485	6.372	7.185
3.0	5.495	6.591	7.556	8.414
4.0	6.150	7.292	8.274	9.132
5.0	6.607	7.763	8.744	9.592

Table 4. Expected number $\mathbb{E}(X(t)|X(t) > 0)$ of the variant type, given it is still present, for various values of c and t, with $p = q = 0.25$, and $X(0) = 1$.

Numerical results for Model C

Loss probability and average time to loss of a variant cell

c	0.1	0.2	0.5	0.8				
l_1	1.0000	1.0000	1.0000	0.9928				
$\mathbb{E}(T\vert \text{ hit } 0)$	1.128	1.201	1.531	2.273				
c	1.0	1.1	1.2	1.5	2.0	3.0	4.0	5.0
l_1	0.9375	0.8838	0.8238	0. 6662	0.5000	0.3333	0.2500	0.2000
$\mathbb{E}(T\vert \text{ hit } 0)$	2.539	2.306	1.977	1.249	0.766	0.440	0.310	0.240

Table 5. Probability that variant cell type goes extinct and mean time to loss of the variant type, conditional on loss, starting from a single variant cell.

Half-life of variant lineage when present

c	0.1	0.2	0.5	0.8				
t_{50}	0.761	0.785	0.869	0.967				
t_{90}	2.630	2.835	3.769	5.743				
c	1.0	1.1	1.2	1.5	2.0	3.0	4.0	5.0
t_{50}	0.950	0.888	0.812	0.617	0.434	0.273	0.199	0.157
t_{90}	6.501	5.881	5.009	3.128	1.885	1.059	0.738	0.567

Table 6. Half-life of variant cell type, conditional on loss, starting from a single variant cell, for various values of c.

Average number of variant stem cells when present

	time t			
c	4.5	6.0	7.5	9.0
0.1	0.0208	0.0060	0.0017	0.0005
0.2	0.0292	0.0098	0.0033	0.0011
0.5	0.0711	0.0366	0.0194	0.0105
0.8	0.1427	0.1008	0.0747	0.0574
1.0	0.2041	0.1656	0.1409	0.1238
1.1	0.2370	0.2024	0.1804	0.1654
1.2	0.2706	0.2405	0.2221	0.2099
1.5	0.3692	0.3529	0.3446	0.3401
2.0	0.5053	0.5016	0.5005	0.5001
3.0	0.6667	0.6667	0.6667	0.6667
4.0	0.7500	0.7500	0.7500	0.7500
5.0	0.8000	0.8000	0.8000	0.8000

Table 7. Probability $\mathbb{P}(X(t) > 0)$ that the variant type is still present with $p = q = 0.25$, and $X(0) = 1$.

	time t			
c	4.5	6.0	7.5	9.0
0.1	1.125	1.129	1.130	1.131
0.2	1.282	1.295	1.300	1.303
0.5	2.039	2.177	2.280	2.365
0.8	3.474	4.081	4.650	5.203
1.0	4.901	6.038	7.096	8.078
1.1	5.740	7.162	8.445	9.584
1.2	6.642	8.328	9.786	11.006
1.5	9.483	11.624	13.140	14.161
2.0	13.332	14.899	15.571	15.839
3.0	15.817	15.980	15.998	16.000
4.0	15.994	16.000	16.000	16.000
5.0	16.000	16.000	16.000	16.000

Table 8. Expected number $\mathbb{E}(X(t)|X(t) > 0)$ of the variant type, given it is still present with $p = q = 0.25$, and $X(0) = 1$.

References

Moran PAP. 1958. Random processes in genetics. Proc. Camb. Philos. Soc., 54, 60–71.
Tavaré S. 1979. A note on finite homogeneous continuous-time Markov chains. Biometrics, 35, 831–834.

R code for calculations

In all cases, we set $N = 16$ and $\tau = 1$.

Numerical results for Model B

The following code computes the probability that the variant lineage is lost, and the mean time to loss, conditional on loss occurring. See Table 1.

```
qmatB <- function(n,c,tau,p,q){
  lam <-rep(0,n)
  mu <- rep(0,n)
  for(i in 2:n) {
    lam[i] <- (i - 1)*(n - i + 1) * tau * c * (p+q)
        / (n - i + 1 + (i-1) * c)
    mu[i] = lam[i]
  }
  qm <-matrix(0,nrow = n+1,ncol = n+1)
  for(j in 2:n) {
    qm[j,j-1] <- mu[j]
    qm[j,j+1] <- lam[j]
    qm[j,j] <- -lam[j] - mu[j]
  }
  return(qm)
}

MmatB <- function(n,c,tau,p,q){
  qm <- qmatB(n,c,tau,p,q)
  q0vec <- matrix(nrow = n-1,ncol = 1)
  q0vec <- qm[2:n,1]
  q0m <- matrix(0,nrow = n-1,ncol = n-1)
  q0m <- qm[2:n,2:n]
  m <- solve(-q0m)
  l <- m %*% q0
```

```
  e0t <- 0
  for (j in 1:(n-1)) e0t <- e0t + m[1,j]*l[j]
  e0t <- e0t/l[1]   # starting from 1 variant cell
  return(c(c,e0t,l[1]))
}
```

The following code computes the values of t_{50} and t_{90} for new variant lineage. See Table 2.

```
find0probB <-function(eps,i,n,c,tau,p,q){
  qm1 <- qmatB(n,c,tau,p,q)
  x <- 1
  dev <- 1.0 # eps < 1 here!
  val1 <- expm(x * qm1)[i+1,1]
  while (dev >= eps){
    x <- 2*x
    val2 <- expm(x * qm1)[i+1,1]
    dev <- val2 - val1
    val1 <- val2
  }
  return(val1)
}

funB <- function(x,n,c,tau,p,q){
  prob <- find0probB(0.00001,1,n,c,tau,p,q)
  qm1 <- qmatB(n,c,tau,p,q)
  # change 0.5 to get other percentiles
  fval <- expm(x * qm1)[2,1]/prob - 0.5
  return(fval)
}

manyfunB <- function(cvals){  # cvals is a vector of c values
  m <- length(cvals)
  times <-rep(0,m)
  for(i in 1:m){
  tt <- uniroot(funB,c(0,200),n=16,c = cvals[i],tau = 1.0, p=0.25,q = 0.25,
     tol = 0.0001)
   times[i] <- tt$root
  }
  return(times)
```

```
}
```

The following code computes the probability that the variant type is present, $\mathbb{P}(X(t) > 0)$, and expected number $\mathbb{E}(X(t)|X(t) > 0)$ of the variant type, given it is still present. See Tables 3 and 4.

```
library(expm)

hitTime <- function(t,n,cvals,tau,p,q){
  nc<- length(cvals)
  df <- matrix(0,ncol=3,nrow = nc)
  for(j in 1:nc){
  c <- cvals[j]
  qm1 <- qmatB(n,c,tau,p,q)
  pt <- expm(t * qm1)
  # print(pt[2,])
    df[j,1] <- c
    df[j,2] <- 1.0 - pt[2,1] # starting from 1 variant cell
    tot <- 0.0
    for(l in 1:n) {tot <- tot + l * pt[2,l+1]}
    df[j,3] <- tot/df[j,2]
  }
  return(df)
}
```

Numerical results for Model C

The following code computes the probability that the variant lineage is lost, and the mean time to loss, conditional on loss occurring. See Table 4.

```
qmatC <- function(n,c,tau){
  lam <-rep(0,n)
  mu <- rep(0,n)
  for(i in 2:n) {
    lam[i] <- (i - 1)*(n - i + 1) * tau * c / n
    mu[i] = (i - 1)*(n - i + 1) * tau / n
  }
  qm <-matrix(0,nrow = n+1,ncol = n+1)
  for(j in 2:n) {
    qm[j,j-1] <- mu[j]
    qm[j,j+1] <- lam[j]
```

```
    qm[j,j] <- -lam[j] - mu[j]
  }
  return(qm)
}

MmatC <- function(n,c,tau){
  qm <- qmatC(n,c,tau)
  q0vec <- matrix(nrow = n-1,ncol = 1)
  q0vec <- qm[2:n,1]
  q0m <- matrix(0,nrow = n-1,ncol = n-1)
  q0m <- qm[2:n,2:n]
  m <- solve(-q0m)
  l <- m %*% q0vec
  e0t <- 0
  for (j in 1:(n-1)) e0t <- e0t + m[1,j]*l[j]
  e0t <- e0t/l[1] # starting from 1 variant cell
  return(c(c,e0t,l[1]))
}
```

The following code computes the values of t_{50} and t_{90} for new variant lineage. See Table 6.

```
find0probC <-function(eps,i,n,c,tau){
  qm1 <- qmatC(n,c,tau)
  x <- 1
  dev <- 1.0 # eps < 1 here!
  val1 <- expm(x * qm1)[i+1,1]
  while (dev >= eps){
    x <- 2*x
    val2 <- expm(x * qm1)[i+1,1]
    dev <- val2 - val1
    val1 <- val2
  }
  return(val1)
}

funC <- function(x,n,c,tau){
  prob <- find0probC(0.00001,1,n,c,tau)
  qm1 <- qmatC(n,c,tau)
  # change 0.5 below to get other percentiles
  fval <- expm(x * qm1)[2,1]/prob - 0.5
```

```
  return(fval)
}

manyfunC <- function(cvals){ # cvals is a vector of c values
  m <- length(cvals)
  times <-rep(0,m)
  for(i in 1:m){
  tt <- uniroot(funC,c(0,100),n=16,c = cvals[i],tau = 1.0, tol = 0.0001)
   times[i] <- tt$root
  }
  return(times)
}
```

The following code computes the probability that the variant type is present $\mathbb{P}X(t) > 0)$, and expected number $\mathbb{E}(X(t)|X(t) > 0)$ of the variant type, given it is still present. See Tables 7 and 8.

```
library(expm)

# cvals is a vector of c-values

hitTimeC <- function(t,n,cvals,tau,p,q){
  nc<- length(cvals)
  df <- matrix(0,ncol=3,nrow = nc)
  for(j in 1:nc){
    c <- cvals[j]
    qm1 <- qmatC(n,c,tau,p,q)
    pt <- expm(t * qm1)
    df[j,1] <- c
    df[j,2] <- 1.0 - pt[2,1] # starting from 1 variant cell
    tot <- 0.0
    for(l in 1:n) {tot <- tot + l * pt[2,l+1]}
    df[j,3] <- tot/df[j,2]
  }
  return(df)
}
```

Supplementary Note B

Evidence that a solitary FC patch is derived from a single founder FC

When we observed a solitary FC patch in the analyses of single FSC lineages we asserted that it was founded by a single FC (as in Figure S4A). Might it be possible that those patches were instead founded by two FCs of the same genotype that differentiated immediately after the “symmetrical” division of their parent FSC? First, we consider whether all FCs might be specified in this manner (Figure S4B). In this scenario, an unpaired FC patch cannot be formed in the first cyst populated by twin-spot derivatives of recombination in an FSC. Consequently, our observation of unpaired FC patches in egg chamber 4 would require that there were at least seven cycles of cyst population with FCs in 72h, which is not compatible with a 12h egg chamber budding cycle under optimal conditions. The second possibility is that all solitary FC patches we observed result from two FC daughters of an FSC, while some other, non-solitary FC patches (those accompanied by FSCs, ECs or additional FC patches of the same lineage) derive from a single founder FC, produced, for example, by an FSC division with an asymmetric outcome (Figure S4C). In this scenario, the asymmetric FSC divisions could produce unpaired FC patches in the first cyst populated after recombination in FSCs (compatible with such patches in egg chamber 4 and the expected time-line of six cycles of FC contribution over 72h). However, the size of all solitary FC patches (with no other matching FSC, EC or FC), always founded by two FCs, should be larger than the average of all other FC patches, frequently founded by only one FC. In our two twin-spot experiments, the average percentage of the FC epithelium of an egg chamber occupied by solitary FC patches prior to egg chamber 4 (19.0%, n= 10 and 16.5%, n= 31) was not larger than the average contribution for all FC patches (18.9%, n= 28 and 18.8%, n= 63 for B, G and BG patches). We can therefore be confident that the delayed solitary FC patches we observed in our twin-spot experiments were not founded by two FCs of the same color (as in Figure S4B or S4C). Instead, we can confirm the prior assertion that an FSC daughter marked shortly after heat-shock

often remained in the germarium for 1-5 cycles and then became a single FC without any intervening divisions (as in Figure S4A).

Supplementary Note C

Selection and verification of lineages derived from a single FSC

To infer the past behavior of a parent FSC it is important to determine whether each lineage of a given color derived from a single cell. We recognized two circumstances, accounting for 29 of the 78 lineages examined, where there was almost certainly more than one cell of origin for a specific color. First, when an ovariole contained two overlapping twin-spot pairs (for example, G:BR and BR:GR) we acknowledged that the common lineage color (BR in this example) derived from (at least) two cells. Second, we assigned two cells as the source of a pair of twin-spot colors (e.g. two FSCs yielding two pairs of BR:GR daughters) whenever the number of ECs, FCs and FSCs of one color exceeded what could be produced from a single cell if an FSC can divide no more than one time per cycle of egg chamber budding. We expected that the vast majority of the remaining 49 lineages should have originated from single cells because we used a very mild heat-shock to induce clones. We tested this expectation by measuring the contribution of each lineage to the entire FC epithelium available to marked FSC derivatives (spanning 3 or 4 egg chambers in each ovariole). The mean contribution from these 49 lineages was 7.6%, or roughly 1/13 of all FCs. There are thought to be about 14-16 FSCs per germarium, so an average contribution of 1/13 of all FCs is consistent with the conclusion that almost all of these 49 lineages derive from a single FSC.

Supplemental Figure Legends

Figure S1. Outcomes for competition of a variant stem cell lineage amongst normal neighbors.
A population of sixteen stem cells (circles) initially has one variant (green) carrying a genetic modification. Over time (downward arrows) there are many possible stochastic trajectories, wherein variant stem cells can duplicate or be lost at each step. The probabilities of different outcomes were derived mathematically from quantitative models of different stem cell organizations (Figure 1 and Supplementary Note A). These include the probability that the variant lineage is the "winning" lineage when only a single lineage remains (bottom row) and, at earlier, intermediate times, the probability that the variant lineage is still present, the expected number of stem cells per surviving variant lineage, and hence the expected number of variant stem cells in the population.

Figure S2. Single-channel images for twin-spot lineage analysis.
(A-H) FSC twin-spot clone 72h after induction from Fig. 3A, B to show single-color images. Section z4 from Fig. 3A is shown in (A) together with (C) blue (β-galactosidase staining) and white (Fas III staining), (E) green (GFP) and (G) red (RFP) channels only. Section z6 from Fig. 3B is shown in (B) together with (D) blue (β-galactosidase staining), (F) green (GFP) and (H) red (RFP) channels only. Scale-bar is 20μm. Diagonal white lines indicate edges of original images.

Figure S3. Division-independent differentiation of FSCs to FCs.
(A-D) Analysis of multi-color FSC twin-spot clones 72h after induction, as described for Figure 3. (A) The germarium and first three egg chambers (ECh 1-3) and (B) fourth egg chamber (ECh 4) of the ovariole illustrated in (C). (A-C) Egg chamber 4 contains two pairs of twin-spot clones (BR:GR, BG:BGR), with no unpaired patch. It is therefore likely that these FCs derived from recombination in FCs. Since many ovarioles from the same experiment did include contributions of marked FSC daughters to ECh 4, we

reasoned that egg chamber production was likely synchronous to within one cycle in all ovarioles and hence that derivatives from recombination in an FSC extend up to egg chamber 3 for this ovariole (and all others with no definitive contribution to ECh 4). There is only one B FC patch, no B FSC and no B ECs, implying that there were no divisions of the B daughter of the FSC after it was born (shortly after the time of heat-shock). The B (blue) FC patch is in the germarium, implying that the B daughter of the FSC initially undergoing recombination (to produce a B and GR twin-spot) became an FC only after at least 3 cycles (after the opportunity to populate ECh 3, 2 and 1), at least 36h after it was born, confirming that an FSC daughter can become an FC long after it is born. Scale-bar is 20μm. (C) The inferred histories of the B:GR and BR:GR twin-spot daughter pairs derived from two separate FSCs are illustrated from immediately after recombination marking (bottom) through each cycle of egg chamber budding up to the final stained ovariole at 72h after heat-shock. In cycle 2, a GR (yellow) and a BR (purple) FSC become founder FCs (which eventually form patches in ECh 2 of the final stained ovariole). Other FC founders are produced in cycle 3 (1 BR + 1 GR, ending up in ECh 1), cycle 4 (B, ending up in the posterior germarium) and cycle 5 (1BR + 1GR, ending up as the most anterior Fas3-positive cells of the germarium). (D) The total number of founder FCs and ECs produced in each cycle from the original four FSC daughters are tabulated, together with the inferred number of marked FSCs present during each cycle. In keeping with our finding of division-independent differentiation, each cycle was split into a phase where FSCs can first divide and then FSCs can become FCs or ECs (cartoons show the end of each labeled cycle except for the bottom cartoon, which represents the middle of cycle 1). By modeling each cycle with division first, the number of FSCs is artificially inflated in the middle of a cycle. The number of marked FSCs at the start (s; before FSC division), middle (m; after FSC division but prior to FC or EC formation) and end (e; after FC or EC production) of each cycle is recorded. Some FSC division cycles can be deduced with certainty, while others are assigned to space out FSC divisions evenly (e.g. to produce FCs in cycles 2, 3, 4 and 5, and leave two FSCs, the BR FSC must divide in cycle 1 and at least one of the two BR FSCs must divide in each subsequent cycle). The cartoons and tabulation represent a single “best-guess” history. To calculate FSC division frequency for the marked cells (of all colors) over the entire time period shown we summed the number of marked FSCs

present at the start of each cycle (24 over 5 cycles) and the number of marked FSC divisions (11 over 5 cycles). Overall, there were 11 FSC divisions out of 24 opportunities to divide, yielding an FSC division frequency of 11/24. FSC differentiation frequency was calculated in a similar manner. We summed the number of marked FSCs present at the middle of each cycle (35 over 5 cycles) and the number of FCs (9) and ECs (0) produced from those FSCs to calculate an FSC differentiation frequency of 9/35 for FCs and 0/35 for ECs, Analogous data and tabulation of inferred "best-guess" FSC histories from all ovarioles were used to calculate average FSC division frequency (total number of marked FSC divisions divided by total number of FSCs available for division) and differentiation frequency (total number of marked FCs or ECs divided by total number of FSCs available for differentiation).

Figure S4. Solitary FC patches are founded by single FCs.

(A-C) Depiction of hypothetical outcomes for three models of FC production (see Supplementary Note B). In each case, cycle 1 depicts two pairs of daughters (B, GR and G, BR) derived from mitotic recombination in two FSCs immediately after their formation and first opportunity to become a FC. FSCs are drawn to the left, and new FCs are drawn immediately to the right (posterior) of the Fas3 (red) staining border, adjacent to a flattened stage 2b germline cyst. The next cycle shows any new FCs from cycle 1 duplicated and associated with a rounded stage 3 germarial cyst, as well as any new FCs (immediately posterior to Fas3) or FSCs (anterior to Fas3). The third diagram depicts ovaries 72h after heat-shock, including in each case an unpaired FC patch in egg chamber 4 (as observed in several ovarioles), a solitary FC patch (no other cells of this color) and one or more additional FC patches. (A) An FSC always divides to produce two FSCs and an FSC can become an FC at any time. The BR FSC daughter immediately becomes an FC and therefore appears in egg chamber 4 after the next 5 cycles (6 cycles in total). The B FSC does not divide and becomes an FC three cycles later. Each FC patch is founded by a single FC of a given color. (B) FCs are always formed by symmetric division of an FSC. Both G and BR FSC daughters immediately become FCs. The GR FSC daughter divides in the next cycle to form two GR FCs. Because the earliest time an

unpaired twin-spot (GR) is founded is in the second cycle, the appearance of unpaired FCs in egg chamber 4 requires that daughters of recombination in an FSC first contributed to egg chamber 5 (as shown for G, BR daughters) and there must therefore have been 7 cycles after heat-shock (too many for 72h). All FC patches derive from two founder FCs in this model. (C) FCs always form at the time of FSC division, sometimes in pairs but other times as a single FC (plus FSC sister). One FSC parent first produces a G FSC and a BR FC, resulting in an unpaired FC patch in egg chamber 4 five cycles later (total of 6 cycles). In the next cycle, the B FSC divides to produce two FCs, resulting in a solitary FC patch; all solitary FC patches must derive from two founder FCs in this model. The G FSC divides with asymmetric outcomes (1 FSC + 1 FC) in cycle 2 and cycle 3, and then it divides symmetrically to produce two FSCs. The G FC patches are each founded by a single FC. Solitary FC patches are larger than non-solitary patches on average because they are all founded by two FCs of the same color.

Figure S5. Measurement of competition for variant marked FSCs

FSC clones (green) of control or mutant genotypes, generally initiating in one or two FSCs, are induced through heat-shock induction of *hs-flp* recombinase. After 6d or 12d (downward arrows), many germaria are scored (four examples are shown for each genotype) for the number of marked FSCs in order to calculate the average number of marked FSCs per germarium. All germaria contain a fixed number of total (marked plus unmarked) FSCs at all times. For control FSCs (middle column), some germaria lose all marked FSCs, while in others the marked FSC has generally amplified to different degrees. For genotypes where division of the marked FSC is slower than for neighbors (left column), marked FSCs are lost at a greater rate than controls and amplify less frequently, leading to a lower average number of marked FSCs (Figure 5). The converse outcome is seen for genotypes where marked FSCs divide faster than their unmarked neighbors (right column), leading to a higher average number of marked FSCs (Figure 5).

Figure S6. Representation of FSCs with altered proliferation rate is not due to changes in EC production, FSC location or FC production.

(A, B) The mean number of FSCs (red) and ECs (blue) per ovariole at (A) 6d and (B) 12d after clone induction, expressed as percentage of control values, is shown for MARCM FSC clones of the listed genotypes. The loss of FSCs for *yki*, *cycE* and *cutlet* cannot be explained by increased EC production and the increase in FSCs for the other genotypes cannot be explained by reduced EC production. Error bars show SEM (6d FSC and EC content: n= 58 (54), 38 (61), 35 (65), 43 (65), 52 (54), 49 (65), 41 (65) ovarioles in order shown, 12d FSC and EC content: n= 58 (69), 53 (55), 37 (71), 56 (71), 63 (69), 56 (71), 54 (71) ovarioles in order shown; values in parentheses are for relevant controls). (C) The proportion of all marked FSCs that are in the most posterior layer (layer 1), expressed as a percentage of control values for MARCM FSC clones, is shown for the indicated genotypes 6d (blue) and 12d (red) after clone induction. Error bars show SEM (6d: n= 64 (158), 61 (205), 75 (159), 173 (159), 201 (158), 193 (159), 141 (159) FSCs in order shown, 12d: n= 49 (274), 38 (176), 60 (249), 369 (249), 403 (274), 313 (249), 338 (249) FSCs in order shown; values in parentheses are for relevant controls). (D) The proportion of all ovarioles with at least one marked FSC that includes at least one marked FC patch, expressed as a percentage of control values for MARCM FSC clones, is shown for the indicated genotypes 6d (blue) and 12d (red) after clone induction. Error bars show SEM (6d: n= 19 (44), 21 (39), 27 (42), 43 (42), 48 (44), 36 (42), 39 (42) FSC clones in order shown, 12d: n= 33 (53), 30 (34), 24 (53), 29 (53), 41 (53), 30 (53), 20 (53) FSC clones in order shown; values in parentheses are for relevant controls).

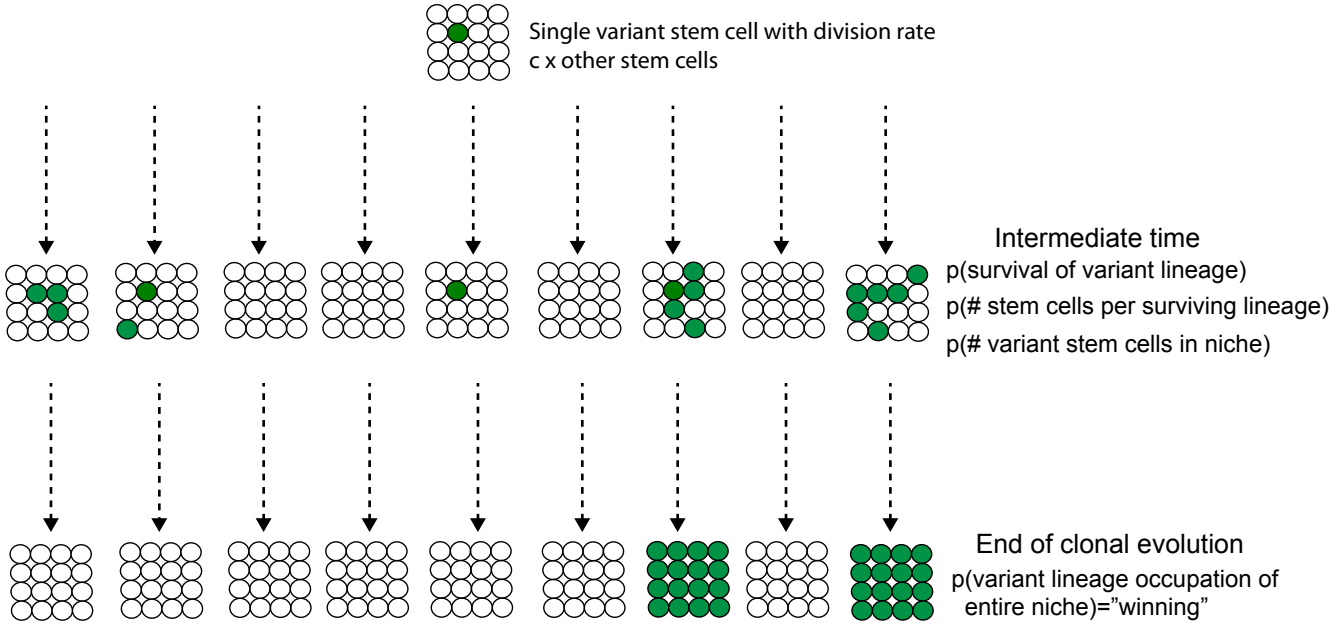

Single variant stem cell with division rate
c x other stem cells
Intermediate time
p(survival of variant lineage)
p(# stem cells per surviving lineage)
p(# variant stem cells in niche)
End of clonal evolution
p(variant lineage occupation of
entire niche)="winning"

Figure S1

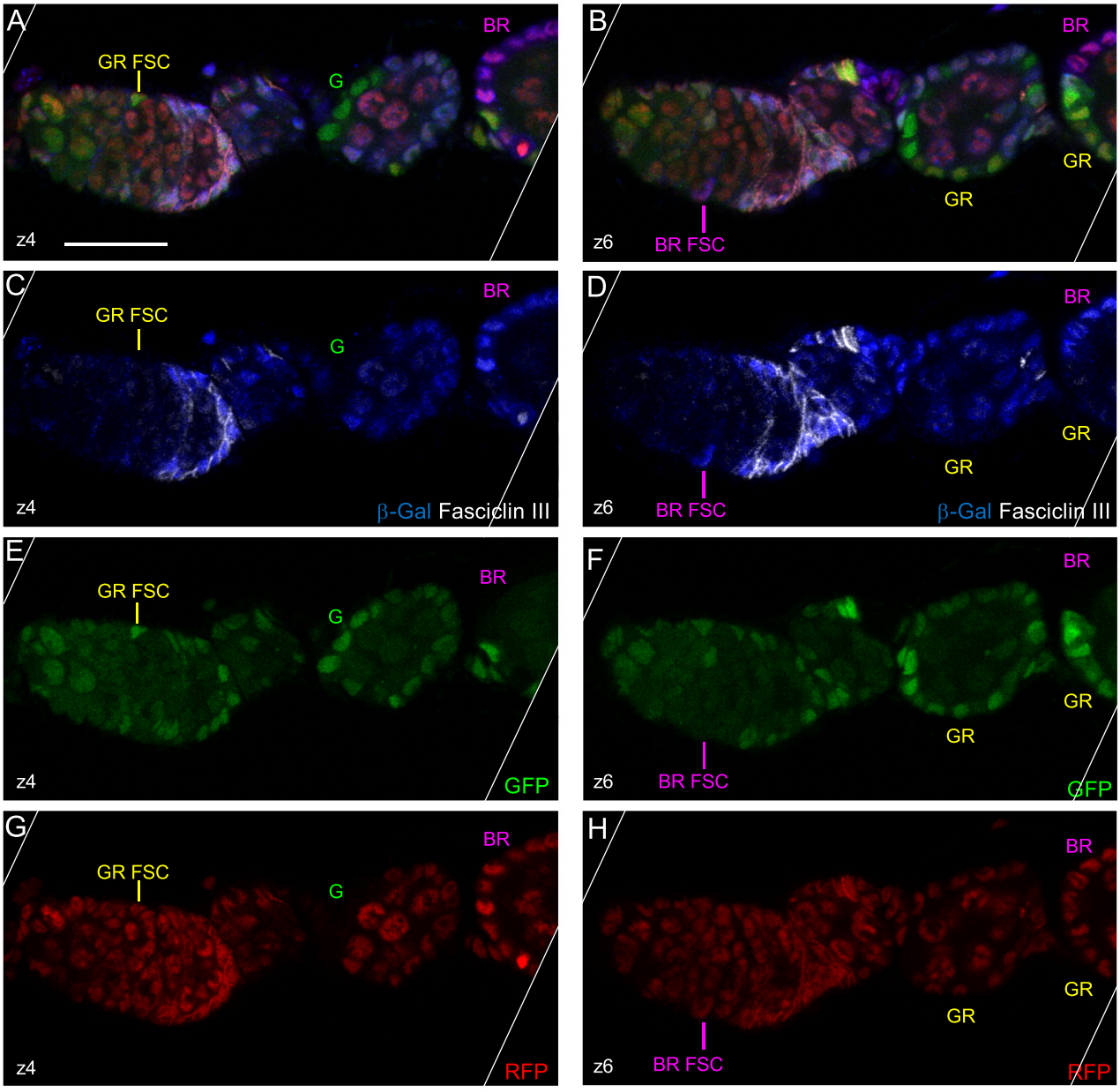
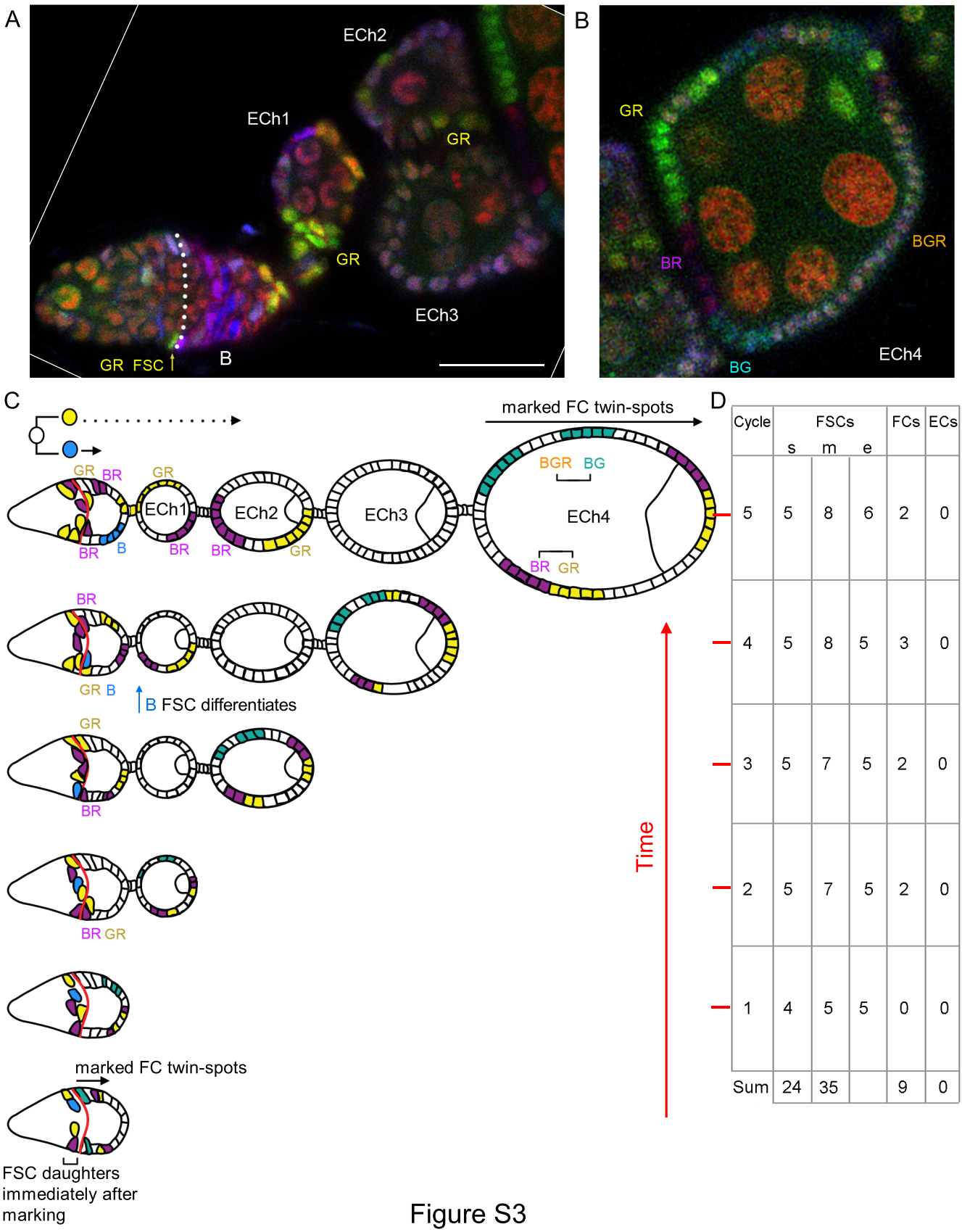

A
GR FSC
G
BR
z4
B
BR
GR
GR
BR FSC
z6
C
GR FSC
G
BR
z4
β-Gal Fasciclin III
D
BR
GR
GR
BR FSC
z6
β-Gal Fasciclin III
E
GR FSC
G
BR
z4
GFP
F
BR
GR
GR
BR FSC
z6
GFP
G
GR FSC
G
BR
z4
RFP
H
BR
GR
GR
BR FSC
z6
RFP

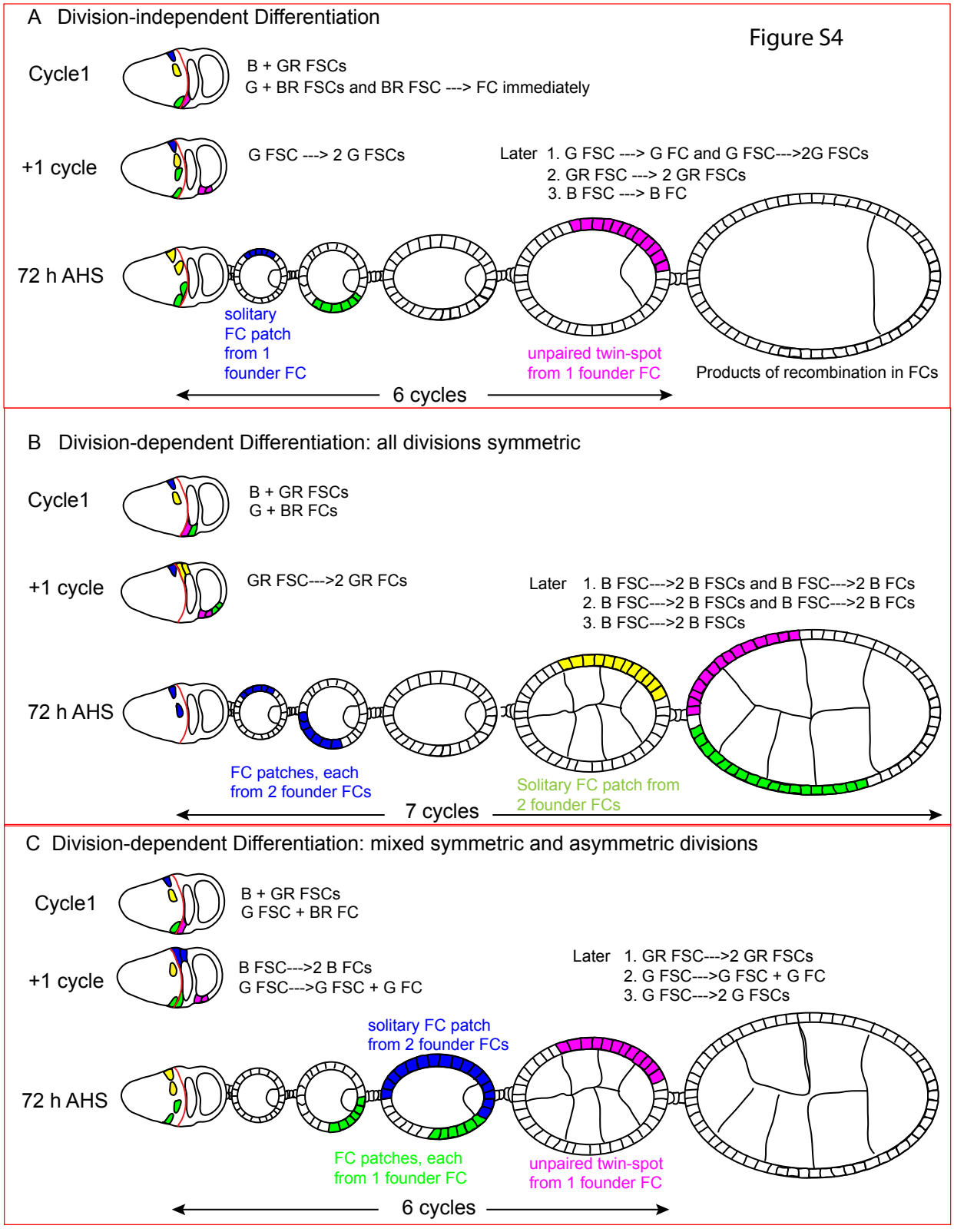
Figure S2



Cycle	FSCs s	FSCs m	FSCs e	FCs	ECs
5	5	8	6	2	0
4	5	8	5	3	0
3	5	7	5	2	0
2	5	7	5	2	0
1	4	5	5	0	0
Sum	24	35		9	0

Figure S3

Figure S4


A Division-independent Differentiation
Cycle1
B + GR FSCs
G + BR FSCs and BR FSC ---> FC immediately
+1 cycle
G FSC ---> 2 G FSCs
Later 1. G FSC ---> G FC and G FSC--->2G FSCs
2. GR FSC ---> 2 GR FSCs
3. B FSC ---> B FC
72 h AHS
solitary FC patch from 1 founder FC
unpaired twin-spot from 1 founder FC
Products of recombination in FCs
6 cycles
B Division-dependent Differentiation: all divisions symmetric
Cycle1
B + GR FSCs
G + BR FCs
+1 cycle
GR FSC--->2 GR FCs
Later 1. B FSC--->2 B FSCs and B FSC--->2 B FCs
2. B FSC--->2 B FSCs and B FSC--->2 B FCs
3. B FSC--->2 B FSCs
72 h AHS
FC patches, each from 2 founder FCs
Solitary FC patch from 2 founder FCs
7 cycles
C Division-dependent Differentiation: mixed symmetric and asymmetric divisions
Cycle1
B + GR FSCs
G FSC + BR FC
+1 cycle
B FSC--->2 B FCs
G FSC--->G FSC + G FC
Later 1. GR FSC--->2 GR FSCs
2. G FSC--->G FSC + G FC
3. G FSC--->2 G FSCs
solitary FC patch from 2 founder FCs
72 h AHS
FC patches, each from 1 founder FC
unpaired twin-spot from 1 founder FC
6 cycles

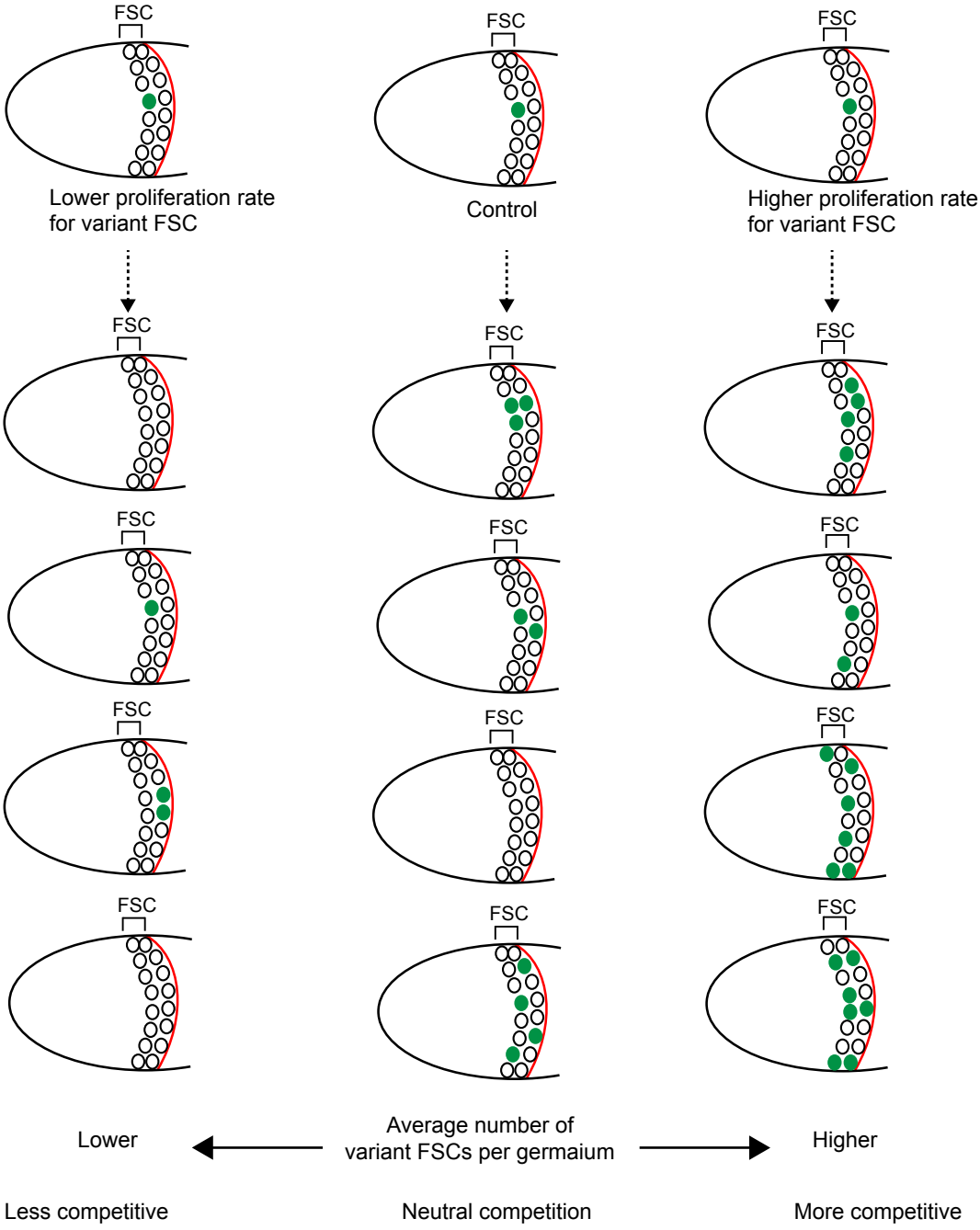

FSC
Lower proliferation rate
for variant FSC
FSC
Control
FSC
Higher proliferation rate
for variant FSC
Lower
Average number of
variant FSCs per germaium
Higher
Less competitive
Neutral competition
More competitive

Figure S5

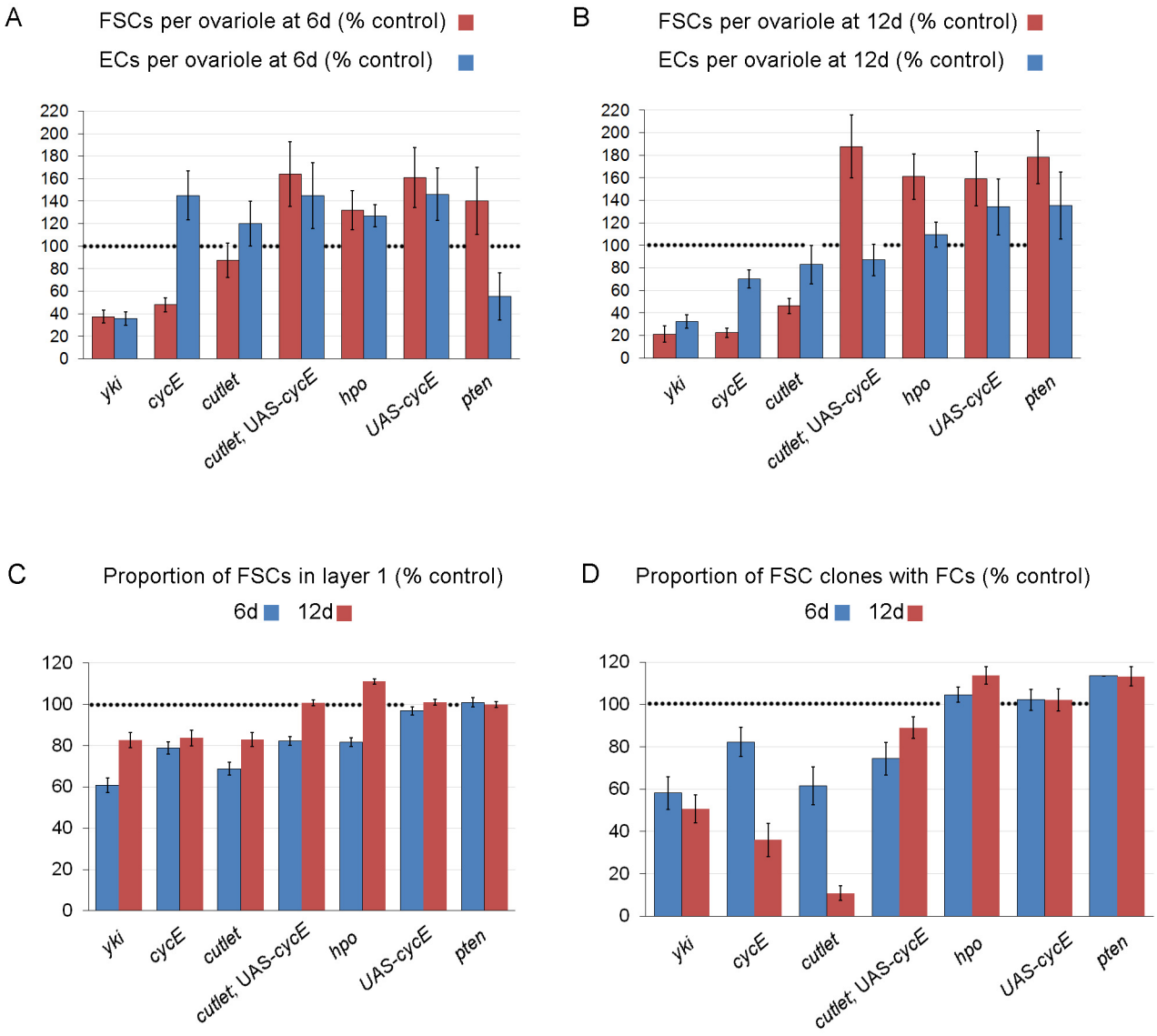

A
FSCs per ovariole at 6d (% control)
ECs per ovariole at 6d (% control)
B
FSCs per ovariole at 12d (% control)
ECs per ovariole at 12d (% control)
C
Proportion of FSCs in layer 1 (% control)
6d
12d
D
Proportion of FSC clones with FCs (% control)
6d
12d
yki
cycE
cutlet
cutlet; UAS-cycE
hpo
UAS-cycE
pten

Figure S6

Table S1: FSC location, EC and FC production for mutant FSCs with altered division rates

	EdU index of all FSCs at 6d (n)	**Mean # FSCs per ovariole at 12d** (n)	**Mean # ECs per ovariole at 12d** (n)	**Percentage of FSCs in layer 1 at 12d** (n)	**Percentage of FSC clones with FCs* at 12d** (n)
Control	**0.29** (158)	**3.97** (69)	**1.81** (69)	**51.8** (274)	**62.3** (53)
yki	**0.031** (64)	**0.84** (58)	**0.59** (58)	**42.9** (49)	**31.6** (19)
hpo	**0.39** (201)	**6.4** (63)	**1.98** (63)	**57.6** (403)	**70.8** (48)
Control	**0.28** (205)	**3.2** (55)	**1.4** (55)	**47.2** (176)	**79.4** (34)
cycE	**0.049** (61)	**0.72** (53)	**0.98** (53)	**39.5** (38)	**28.6** (21)
Control	**0.23** (159)	**3.51** (71)	**0.94** (71)	**52.2** (249)	**67.9** (53)
cutlet	**0.093** (75)	**1.62** (37)	**0.78** (37)	**43.3** (60)	**7.4** (21)
cutlet; UAS-cycE	**0.39** (173)	**6.59** (56)	**0.82** (56)	**52.6** (369)	**60.5** (42)
UAS-cycE	**0.40** (193)	**5.59** (56)	**1.27** (56)	**52.7** (313)	**69.4** (36)
pten	**0.52** (141)	**6.26** (54)	**1.28** (54)	**52.1** (338)	**76.9** (39)

* FCs scored in germarium and first four egg chambers