Estimation of patch forming efficiency

Long-term patch-forming efficiencies of X, Z, and Ngn3$^+$ A_{undiff} during homeostasis (summarized in Figure 2F) were calculated as the approximate percentages of the number of clonal patches that survived over the long term (3 months after pulse) per testis normalized by the number of initially labeled cells (2 days after pulse) per testis. Total cell numbers used for these calculations (i.e., that of GFRα1$^+$, GFRα1$^-$, and total A_{undiff} per testis) were drawn from published data based on immunostained whole-mount seminiferous tubules of adult C57B/6 mice (Hara et al., 2014; Nakagawa et al., 2010). Namely, the rounded numbers of GFRα1$^+$ cells (3×10^1 per mm tubule length) and the length of seminiferous tubules (2×10^3 mm per testis) lead to an estimate of approximately 6×10^4 GFRα1$^+$ cells per testis. Further, given the ratio of GFRα1$^+$ and GFRα1$^-$ A_{undiff}, which was found to be roughly 1 to 2, the numbers of GFRα1$^-$ and total A_{undiff} were estimated to be around 1.2×10^5 and 1.8×10^5, respectively, per testis.

For X (Plvap$^+$) cells, 2 days after injecting two consecutive doses of 2.0 mg 4OH-Tamoxifen, with a 7 hour time interval, into adult *Plvap^{CreERT2};CAG-CAT-EGFP* mice, proportions of labeled (GFP$^+$) cells out of the total GFRα1$^+$ cells were calculated in immunostained whole-mount seminiferous tubules prepared from 3 different individuals, each of which was long enough to cover multiple waves of seminiferous epithelium; this fraction was then multiplied by the total number of GFRα1$^+$ cells per testis to obtain the number of initially labeled X cells. Then, the number of long-term patches of GFP$^+$ cells per testis 3 months after pulse-labelling using the same protocol were counted in 5 testes from different mice. Finally, the patch-forming efficiency was calculated from these numbers. Actual counts are summarized below.

Calculation of patch-forming efficiency of X cells

2 days after pulse labeling using PlvapCreER

exp.	number of GFP$^+$ cells [a]	number of GFRα1$^+$ cells [b]	GFP$^+$ cells/GFRα1$^+$ cells (mean ± SD)[c]		estimated number of GFP$^+$ cells/testis [d]
1	86	4188	0.021	(0.020 ± 0.002)	$1.2 \pm 0.1 \times 10^3$
2	48	2164	0.022		
3	31	1774	0.017		

3 months after pulse labeling using PlvapCreER

exp.	number of total patches (mean ± SD)[e]		patch-forming efficiency [f]
4	261	(250 ± 35)	$2.1 \pm 0.3 \times 10^{-1}$
5	239		
6	203		
7	251		
8	299		

a, b: counts from whole-mount seminiferous tubule specimens

c = a/b

d = c × total number of GFRα1$^+$ A_{undiff} per testis (6×10^4)

f = e/d

The patch-forming efficiency of Ngn3$^+$ A_{undiff} was calculated based on numbers obtained in experiments published in Nakagawa et al. (2010), in which adult *Ngn3-CreER^{TM};CAG-CAT-EGFP* mice were injected with a single dose of 2 mg 4OH-tamoxifen. Calculations were conducted in the same manner as described for X cells (above), except for the use of the entire A_{undiff} defined experimentally as E-Cadherin (E-Cad)$^+$ spermatogonia in place of GFRα1$^+$ cells, to obtain the number of initially labelled cells 2 days after pulse. Actual counts and calculations are summarized below.

Calculation of patch-forming efficiency of Ngn3$^+$ cells
2 days after pulse labeling using Ngn3CreER

exp.	number of GFP$^+$ cells [a]	number of E-Cad$^+$ A_{undiff} [b]	GFP$^+$ cells/E-Cad$^+$ A_{undiff}	(mean ± SD) [c]	estimated number of GFP$^+$ cells/testis [d]
1	954	2293	0.42	(0.30 ± 0.10)	$5.5 \pm 1.8 \times 10^4$
2	838	2188	0.38		
3	1565	5307	0.29		
4	996	5923	0.17		
5	977	3865	0.25		

3 months after pulse labeling using Ngn3CreER

exp.	number of total patches [e]		patch-forming efficiency [f]
6	3	(3.8 ± 1.2)	$7.0 \pm 2.1 \times 10^{-5}$
7	5		
8	4		
9	2		
10	5		
11	4		

a, b: counts from whole-mount seminiferous tubule specimens (Nakagawa et al., 2010)
c = a/b
d = c × total number of A_{undiff} per testis (1.8×10^5)
f = e/d

Finally, to calculate the patch forming efficiency of Z (SOX3$^+$/GFRα1$^+$) cells, a single dose of 0.5 mg 4OH-Tamoxifen was administered to adult *Sox3-CreER^{T2};CAG-CAT-EGFP* mice. To estimate the number of initially labeled Z cells, GFP$^+$/GFRα1$^+$ cells and the total number of GFRα1$^+$ cells were counted in 3 immunostained specimens of long seminiferous tubules 2 days after pulse (each prepared from different individuals). Then the portion of GFP$^+$/GFRα1$^+$ cells out of the total GFRα1$^+$ cells was multiplied by the total number of GFRα1$^+$ cells to obtain an approximate number of GFP$^+$ cells per testis. 3 months after pulse-labeling, the observed patches include both those derived from Z (SOX3$^+$/GFRα1$^-$) cells and from SOX3$^+$/GFRα1$^-$ cells. Therefore, to estimate the number of Z-cell derived patches, the total number of patches per testis observed in 3 individuals was subtracted from the expected number of SOX3$^+$/GFRα1$^-$ cell-derived patches, a value calculated as the product of the number of GFRα1$^-$ A_{undiff} (roughly equivalent to the number of SOX3$^+$/GFRα1$^-$ cells; 1.2×10^5 per testis), the labeling efficiency of SOX3$^+$/GFRα1$^-$ cells 2 days after pulse (virtually 100%; See Figure S3R), and the patch-forming efficiency of Ngn3$^+$ cells, a population largely overlapping with GFRα1$^-$ A_{undiff} or SOX3$^+$/GFRα1$^-$

43 cells (7.0×10^{-5}; see above). This translates to a figure of 8.4, comprising only a minority
44 fraction of the patches derived from the total number of SOX3$^+$ cells (179 ± 20 per testis).

Calculation of patch-forming efficiency of Z cells

2 days after pulse labeling using Sox3CreER

exp.	number of GFP$^+$ cells [(a)]	number of GFRα1$^+$ cells [(b)]	GFP$^+$ cells/GFRα1$^+$ cells (mean ± SD) [(c)]		estimated number of GFP+ GFRα1$^+$ cells/testis [(d)]
1	191	1080	0.18	(0.15 ± 0.03)	$8.8 \pm 1.8 \times 10^3$
2	121	1032	0.12		
3	143	987	0.14		

3 months after pulse labeling using Sox3CreER

exp.	number of total patches (mean ± SD) [(e)]		estimated number of patches derived from Z cells [(f)]	patch-forming efficiency of Z [(g)]
4	179	(179 ± 20)	171 ± 20	$1.9 \pm 0.2 \times 10^{-2}$
5	198			
6	159			

a, b: counts from whole-mount seminiferous tubule specimens

c = a/b

d = c × total number of GFRα1+ cells per testis (6×10^4)

f = e - estimated number of patches derived from SOX3$^+$/GFRα1$^-$ cells (8)

g = f/d