

Supplementary figure legends

Figure S1 : Cell cycle markers label a subset of Zfh1-positive cells.

A. *PCNA-GFP* expression (green, single channel in A') was seen in cells near the hub (arrow) that also expressed Zfh1 (blue, single channel in A''), but absent in other cells (arrowhead).

B. CycB immunofluorescence (green, single channel in B') similarly showed expressing (arrows) and non-expressing cells (arrowhead) in a subset of Zfh1-positive cells (blue, single channel in B''). A'' and B'' show Vasa single channel (red in A and B). The hub is indicated by a dotted line.

Figure S2 : Validation of neutral drift dynamics in CySCs using the fraction of Zfh1-positive cells as a readout

We made CySC MARCM clones labeled with nuclear localization signal (nls) GFP on two distinct chromosome arms. We used *FRT^{40A}* for A,C,E,G,I and *FRT^{42D}* for B,D,F,H,J.

A-D. Clonal analysis, GFP (single channels, A'-D') indicates the clone, Vasa (red) labels germ cells and Zfh1 (blue) CySCs and early cyst cells; the hub is indicated by a dotted line.

GFP-labeled control clones were generated by the MARCM technique and analyzed at 2 (A,B) and 14 dpci (C, D). Although clones were small at 2 dpci (A,B), they varied markedly by 14 dpci (C,D).

E,F. Variation of average size of control clones as a function of time. The data points (boxes) show the mean fraction of Zfh1-positive cells in persisting clones normalized to 13 CySCs.

The black line shows a fit of the neutral drift model to the data using an induction frequency of 18% ($q=0.18$) for *FRT^{40A}* (E) and of 30% ($q=0.3$) for *FRT^{42D}* (F). The dashed orange line represents the predicted clonal evolution if only a single CySC clone were induced with a

time-shift of 3 days in E and 5.5 days in F with the same set of parameters. n=59, 76, 69, 84 for FRT^{40A} at 7, 14, 21, 28 dpci, respectively. n= 48 and 39 for FRT^{42D} at 14 and 28 dpci, respectively. Error bars denote SEM.

G, H. Variation of the fraction of testes with labeled FRT^{40A} (G) or FRT^{42D} (H) control clones as a function of time after clone induction. The points show experimental data and the solid line shows the model. Error bars denote SEM.

I,J. Distribution of persisting clone sizes in wild type testes, expressed as a normalized fraction of Zfh1-positive cells. The boxes show experimental data and lines show the predictions of the model. n=59, 76, 69, 84 for FRT^{40A} at 7, 14, 21, 28 dpci, respectively. n= 48 and 39 for FRT^{42D} at 14 and 28 dpci, respectively. Error bars denote SEM.

Figure S3 : *ptc* and *hpo* mutant cyst cells differentiate normally.

A. Control (A-A'''), *ptc* mutant (B-B''') and *hpo* mutant (C-C''') cyst cells were observed that were negative for Zfh1 (arrows, single channel in A''', B''', C''') with labeled membrane extensions ensheathing spermatogonial cysts. Germ cells are labeled with Vasa (red, single channel in A'', B'', C''). Clones are marked by membrane GFP (single channel A', B', C').

Figure S4 : Validation of biased competition dynamics in *ptc* mutant clones using the fraction of Zfh1-positive cells as a readout

A,B. Clonal analysis, GFP (single channels, A',B') indicates the clone, Vasa (red) labels germ cells and Zfh1 (blue) CySCs and early cyst cells; the hub is indicated by a dotted line. GFP-labeled *ptc* mutant clones were generated by the MARCM technique and analyzed at 2 (A) and 14 dpci (B).

C. Variation of average size of *ptc* mutant clones as a function of time. The data points (boxes) show the mean fraction of Zfh1-positive cells in persisting clones, normalized to 13

CySCs. The black line shows a fit of the neutral drift model, modified to have a bias in favor of the labeled cell, to the data using an induction frequency of 30% ($q=0.3$). The dashed orange line represents the predicted clonal evolution if only a single CySC clone were induced with a time-shift of 5.5 days with the same set of parameters. $n=30, 41, 25, 34$ testes at 7, 14, 21, 28 dpci, respectively. Error bars denote SEM.

D. Variation of the fraction of testes with labeled *ptc* mutant clones as a function of time after clone induction. The points show experimental data and the solid line shows the model. Error bars denote SEM.

E. Distribution of persisting *ptc* mutant clone sizes expressed as a normalized fraction of Zfh1-positive cells. The boxes show experimental data and lines show the predictions of the model. $n=30, 41, 25, 34$ testes at 7, 14, 21, 28 dpci, respectively. Error bars denote SEM.

F. GSC number shown at 14 dpci when control (blue diamonds) or *ptc* mutant (red squares) clones are induced. GSC number decreases when *ptc* mutant clones constitute the majority of the Zfh1-positive population.

Figure S5 : Stat92E expression is unchanged in *ptc* mutant clones

A,B. No increase in Stat92E staining (blue, single channel in A''', B''') was seen in *ptc* mutant CySCs (green, arrows, single channel in A', B') at 7 (A) or 14 (B) dpci. Germ cells are labeled with Vasa (red, single channel in A'', B'').

Figure S6 : Cell competition and cell death cannot account for niche competition phenotypes.

A. Over-expression of the cell-competition inducing factor dMyc did not cause loss of GSCs. dMyc over-expressing CySC clones (green, single channel in A') did not colonize the niche. Vasa is red and Tj is blue.

B. GSC number in testes with control or dMyc-expressing CySC MARCM clones (top) or with negatively-marked control and M^+ clones in a $M/+$ background (bottom) at 14 dpci. No significant change was observed in either case.

C,D. Cleaved Caspase-3 expression in testes with control (C) or *ptc* mutant (D) MARCM clones. Clones are marked with GFP (green, single channel in C', D'), Vasa is red and Cleaved Caspase-3 is blue (single channel in C'', D''). Arrows point to the waste bag, a positive control for Caspase activation (Arama et al., 2007).

E. GSC number at 14 dpci when marked clones were present. Removing one genetic copy of the pro-apoptotic gene *hid* did not rescue the displacement of GSCs by *ptc* mutant CySCs.

Figure S7 : Gain-of-function in cellular growth pathways leads to decreased CySC self-renewal.

A-H. *Pten* mutant or *Tsc1* mutant CySCs did not self-renew. Control CySC clones could be recovered at both 2 and 7 dpci (A,B,E,F, arrows). However, while *Pten* or *Tsc1* mutant CySCs could be recovered at 2 dpci (C,G, arrows), they could not be recovered at 7 dpci (D,H, arrowheads indicate differentiating mutant cyst cells). I. Graphs summarizing data in Supplementary Table S2. See Supplementary Table S2 for "n" values.