Supplementary data

AraC treatment
The protocol using the antimitotic drug cytosine-beta-D-arabinofuranoside (AraC) to deplete Type-C and Type-A cells was modified from Doetsch et al. (1999b). Briefly, adult (3-5m old) TnC heterozygous (control) and homozygous (null) mice were anesthetized and a cannula (Brain Infusion Kit II, Alzet) was fixed on the skull 1mm lateral to bregma. AraC (Sigma) was infused for 4 days onto the surface of the brain (4% AraC in 0.9% NaCl) using a subcutaneously implanted mini-osmotic pump (model 1007D, Alzet). Animals were sacrificed after the end of the infusion (day 0), or after 2, 4 or 6d (n=3/4 per genotype per time-point).

Antibodies and immunostaining
Sections were incubated with a blocking solution (10% normal serum, 0.1% Triton X-100; 1h, RT) and then with the following primary antibodies (1h at RT except if otherwise stated): monoclonal anti-BrdU (Sigma, 1/1000 dilution, overnight at RT); monoclonal anti-Mash1 (F. Guillemot, 1/200, 48h at 4ºC); monoclonal anti-PSA-NCAM (1/100, developed by TM Jessel and J Dodd and obtained from the Developmental Studies Hybridoma Bank, developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences); monoclonal anti-TH (Abcam, 1/500); monoclonal anti-GFAP (Sigma, 1/100); polyclonal anti-GFAP (DAKO; 1/700); polyclonal anti-TnC (A. Faisshner, 1/500); polyclonal anti-PH3 (Upstate, 1/500); polyclonal anti-calretinin (Sigma, 1/100); polyclonal anti-Dcx (Abcam, 1/500); polyclonal anti-Olig2 (Chemicon, 1/1000). Appropriate secondary Alexa-conjugated (Molecular Probes; 1/700; 1h at RT) or biotinylated (Jackson ImmunoResearch Laboratories, 1/100) antibodies were used and nuclei were counterstained with Hoechst (1 µg/mL). For BrdU staining, sections were pre-treated with 2N HCl for 30min at 37ºC. Xgal staining was performed as previously reported (Garcion et al. 2004), followed by immunostaining.

BrdU pulse experiments
SEZ-derived neurospheres were treated with 100nM of AraC for 4 days in order to enrich for slow dividing cells. After removal of AraC, spheres were dissociated and cells were plated at clonal densities in flasks in growth medium containing 20ng/ml
FGF-2 and 20ng/ml EGF. Two different pulses of BrdU were performed: a short 3h pulse and a long cumulative 48h one. At the end of each pulse cells were plated on PDL-coated glass coverslips, fixed in 2% PFA for 15min, immunostained for BrdU as described in Experimental Procedures and the percentage of BrdU-positive cells was calculated. The same percentage of cells was found to be in S phase during the short BrdU pulse in both genotypes. In addition, the same percentage of cells had cycled during the 48h pulse in both genotypes (supplementary figure 4). Furthermore, in order to gain a better insight into the cell cycle kinetics of the long-term pulsed populations we calculated the ratio of cells showing intense BrdU labelling (BrdU high) versus the percentage of cells with light staining (BrdU low). This allows not only the assessment of the total number of cells that have divided during the pulse, but also the fraction of cells that have entered S phase only once during the pulse and thus have not diluted BrdU (BrdU high), or that have re-entered S phase resulting in the dilution of BrdU (BrdU low) (Quinn et al., 2007). As shown in supplementary figure 4, there was no difference in the ratio of BrdU high- BrdU low cells between the TnC heterozygous and null mice.

Regeneration simulation model.

The model is constructed to simulate the regeneration of a TnC +/- and a TnC +/- SEZ after a 4-day treatment with AraC. The initial SEZs were assumed to contain 100 type-C cells and 200 type-B cells (based on SEZ cyto-architecture descriptions found in Doetsch et al., 1999b type-B cell numbers are approximately 2 times higher than the type-C cell numbers). The numbers of Type-C cells surviving the AraC treatment were based on our data (Figure 3) and the decrease in the numbers of type-B cells was again based on data from Doetsch et al., 1999b. Type-B cell division kinetics were based on our in vitro data (see results) and the following assumptions were applied.

i) all dividing type-B cells divided asymmetrically to produce one type-B and one type-C cell.

ii) all dividing type-C cells divided symmetrically every 24h to produce two type-C cells.

iii) the type-B cell kinetics were not influenced by the numbers of TaPs during the simulation time-frame.

Only the first divisions of each cell type were modeled.

The model was constructed using the Microsoft Office Excel software.
Legends to supplementary figures

Supplementary Figure 1:
Characteristic images of the LSN area proximal to the LV from a TnC heterozygous (A) and a TnC null (B) mouse. Sections were immunostained with monoclonal GFAP and Hoechst (nuclear staining). The background tissue has been pseudocoloured with red to enable the visualization of the tissue integrity.

Supplementary Figure 2
(A) Characteristic image of Olig2/PCNA double immunostaining, at the LV area. (B) Graph showing the numbers of Olig2-positive cells following AraC treatment, expressed as a percentage of the non-treated levels. (C) Graph showing the percentage of Olig2-positive cells that were also PCNA-positive at the different time-points after AraC treatment. [*: p<0.005, compared to TnC+/− of the same hemisphere, error bars: SEM]

Supplementary Figure 3
Images showing olfactory bulb glomeruli immunostained for TH and calretinin (A), or Dcx immunostained olfactory bulb plexiform layers from TnC+/− (B) and TnC−/− (C) mice. The results of the analysis are summarized in (D). [nuclei are counterstained with Hoechst]

Supplementary Figure 4
Graphs showing the percentage of BrdU-positive cells in clonally grown SEZ-derived neurosphere cells, pulsed for 48h (A) or 3h (B) with BrdU. In (A) bars represent the percentage of all BrdU-positive cells while the fractions of BrdU high and low cells is illustrated by the different colours (see supplementary data). [error bars: SEM]

Supplementary Figure 5
(A) Table summarizing the simulation model of the regeneration of two hypothetical SEZs (from TnC +/- and -/- mice respectively). It should be noted that only the first division of the different cell types is modelled. (B) Type-B cells were assumed to divide only asymmetrically while type-C cells to divide only symmetrically.
Supplementary References