

## **SUPPLEMENTARY FIGURES LEGENDS, Lagace et al.**

**Supplementary Figure 1.** After tamoxifen-induced recombination *in vivo*, SVZ cells from postnatal nestin-CreER<sup>T2</sup>/R26R-YFP mice form primary and secondary neurospheres *in vitro*. **(a, b)** Neurospheres were evident via brightfield microscopy and many of them were YFP+. **(c)** After one week in culture with mitogens, 36.5±5% of the derived spheres were YFP+. This percentage reflects that many cells non-stem cells can generate neurospheres in the adult (Pevny and Rao, 2003). These other cells therefore likely dilute the proportion of total neurospheres *in vitro* that are YFP+ at the time point examined (12 days post-tamoxifen *in vivo*). A similar percentage of secondary spheres were YFP+, and all cells within the YFP+ spheres were YFP+ (data not shown; n=2, 25 spheres per mouse). **(d)** Equal number of neurospheres was generated from nestin-CreER<sup>T2</sup>/R26R-YFP mice and mice negative for both genes (p>0.05).

**Supplementary Figure 2.** Recombined cells can respond to neurogenic stimuli. Nestin-CreER<sup>T2</sup>/R26R-YFP mice were individually housed with open or locked running wheels for 7 days on the 12<sup>th</sup> day after tamoxifen. **(a,b)** Mice with locked wheels **(a)** had notably fewer YFP+ cells in the dentate gyrus SGZ than mice with open wheels **(b)**. Scale bar=100 µm (a-b). **(c)** Significantly more YFP+ cells were evident in the SGZ in mice that ran on the open wheels (bregma by treatment interaction,  $F_{(11,110)}=2.647$ , p<0.01).

## **SUPPLEMENTARY METHODS**

**Neurosphere assay.** Nestin-CreER<sup>T2</sup>/R26R-YFP mice were utilized for the neurosphere assay 12 days post-tamoxifen, a time point when YFP+ cells are readily visible in the postnatal neurogenic regions (e.g. Figures 1-3). Dissected lateral ventricles were dissociated (Seaberg and van der Kooy, 2002), cell viability assessed by trypan blue exclusion (Sigma), and viable cells plated at 10,000 cells/ml in growth media (either Neurobasal-A (Gibco) media supplemented with B27 (Gibco), or DMEM/F12 (Gibco) media supplemented with N2; both medias augmented with EGF (20 ng/ml) and bFGF (10 ng/ml)). Spheres were counted at day 7 *in vitro*, and spheres were assayed for self-renewal via passaging (Tropepe et al., 1999). Quantification of recombined (YFP+) spheres as percent of total spheres was determined by three independent experiments done in quadruplicate. A minimum of three different fields of view was evaluated at 4x to assess a minimum of 25 neurospheres in each field of view. Genotype did not alter neurosphere formation *in vitro*, as similar number of spheres was generated from mice negative for both genes (n=2-3 per genotype; Supplement Figure 1).

## **SUPPLEMENTARY REFERENCES**

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