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Supplemental Information

Interleukin-6 Regulates Adult Neural Stem Cell Numbers during Normal and Abnormal Post-natal Development

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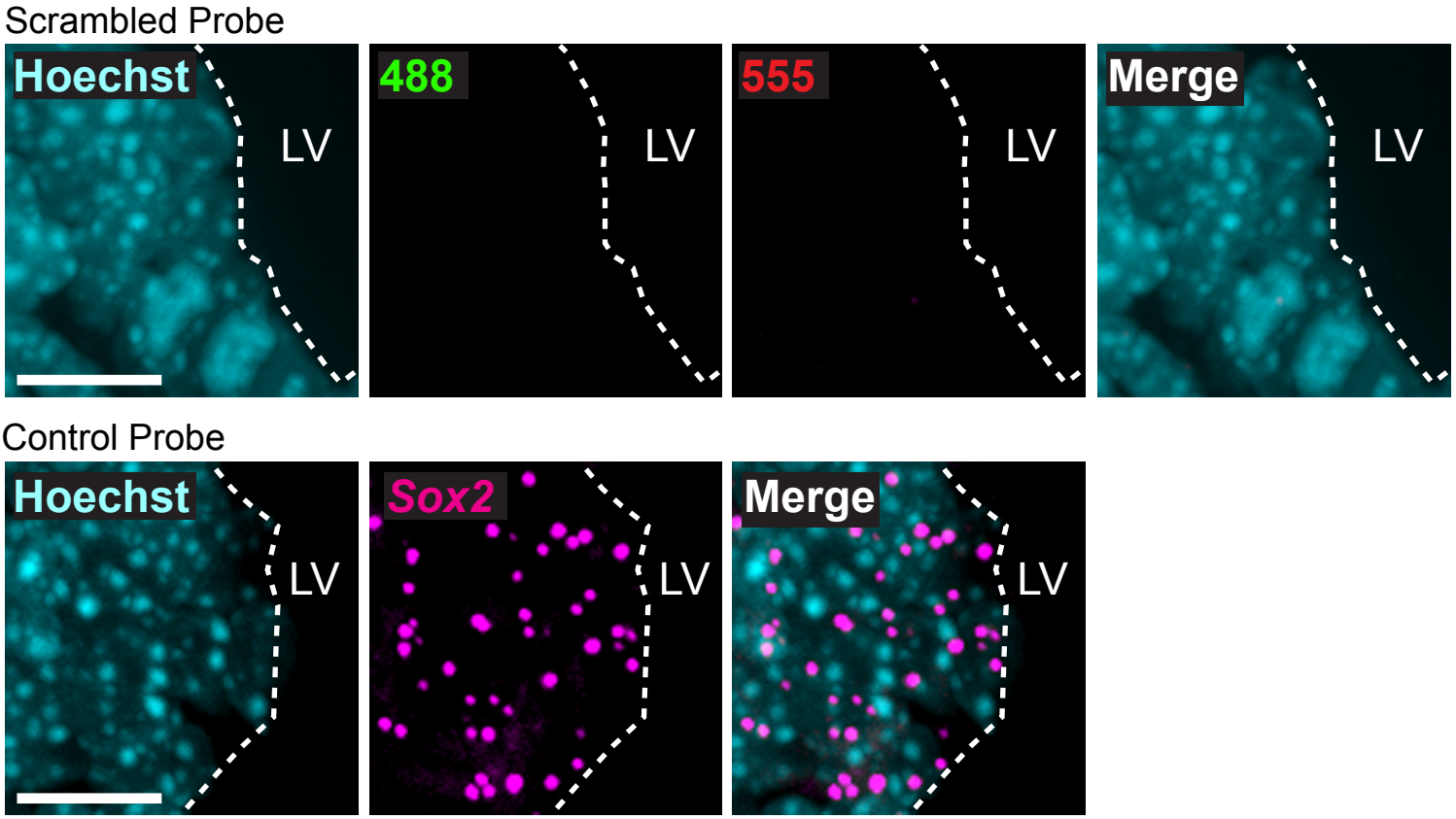

Figure S1. ***Controls for FISH.*** **(Related to Figure 1).** Single molecule FISH analysis of P7 coronal V-SVZ sections hybridized with scrambled negative probes (top panel) tagged with green (488) and red (555) fluorophores, as supplied with the RNAscope kit (Advanced Cell Diagnostics). A Sox2 probe (bottom panel, magenta dots) was used as a positive control. Sections were counterstained with Hoechst 33258 (turquoise in the images). The white-hatched lines indicate the border of the lateral ventricle (LV). Scale bars are 10 μm.

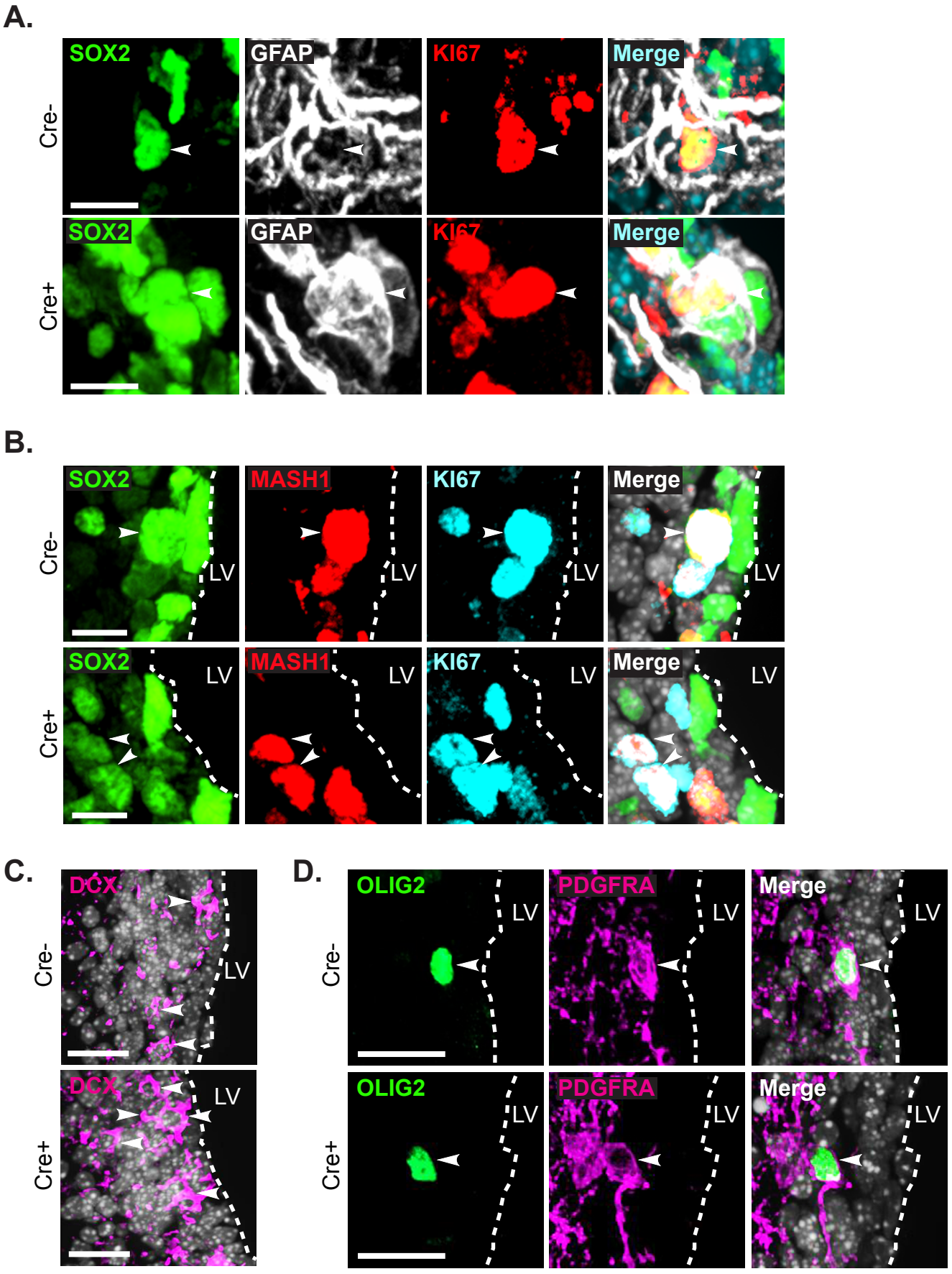

Figure S2. ***Analysis of proliferating and differentiating cells in the perinatal V-SVZ following inducible deletion of IL-6r. (Related to Figure 2).*** High magnification images of the V-SVZ of P8 *Nestin-CreERT2;IL-6r$^{fl/fl}$;R26YFP^{stop}* mice (Cre+) or their *IL-6r$^{fl/fl}$;R26YFP^{stop}* (Cre-) littermates that were exposed to tamoxifen through their mother's milk at P1-P3, given a single injection of BrdU at P7 and analysed one day later. Coronal V-SVZ sections were immunostained in (A) for SOX2 (green), GFAP (white) and KI67 (red), in (B) for SOX2 (green), MASH1 (red) and KI67 (blue), in (C) for DOUBLECORTIN (DCX) (magenta), and in (D) for OLIG2 (green) and PDGFRA (magenta). Merged images show Hoechst 33258 counterstaining (light blue in A and grey in B-D). Arrowheads denote single- (C), double- (D), or triple-labelled cells (A and B). White-hatched lines denote the border of the lateral ventricle (LV). Scale bars are 10 µm (A and B) and 20 µm (C and D).

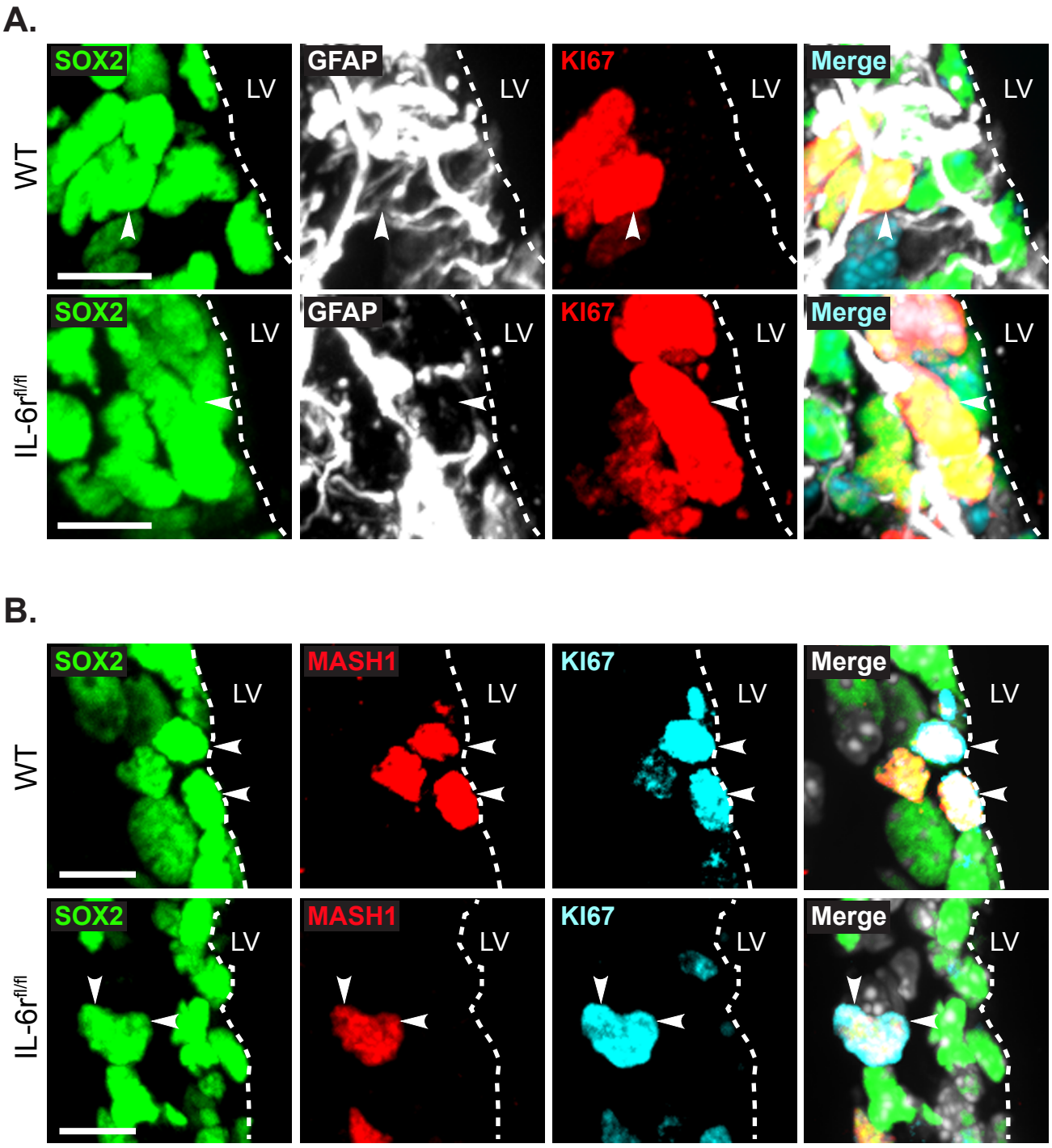

Figure S3. ***Analysis of proliferating NSCs and TA cells in the adult V-SVZ following inducible deletion of IL-6r.*** **(Related to Figure 3).** High magnification images of the V-SVZ of adult *Nestin-CreERT2;IL-6r$^{fl/fl}$;R26YFP^{stop}* (IL-6r$^{fl/fl}$) or *Nestin-CreERT2;IL-6r$^{+/+}$;R26YFP^{stop}* (WT) that were injected daily with tamoxifen for 5 consecutive days from P60 – P64, given a single injection of BrdU at P79 and analysed at P80. In **(A)** sections were immunostained for SOX2 (green), GFAP (white) and KI67 (red), while in **(B)** they were immunostained for SOX2 (green), MASH1 (red) and KI67 (blue). Sections were counterstained with Hoechst 33258 (shown in the right, merged panels as light blue in A and grey in B). Arrowheads denote triple-labelled cells. The white-hatched lines indicate the borders of the lateral ventricle (LV). Scale bars are 10 μm.

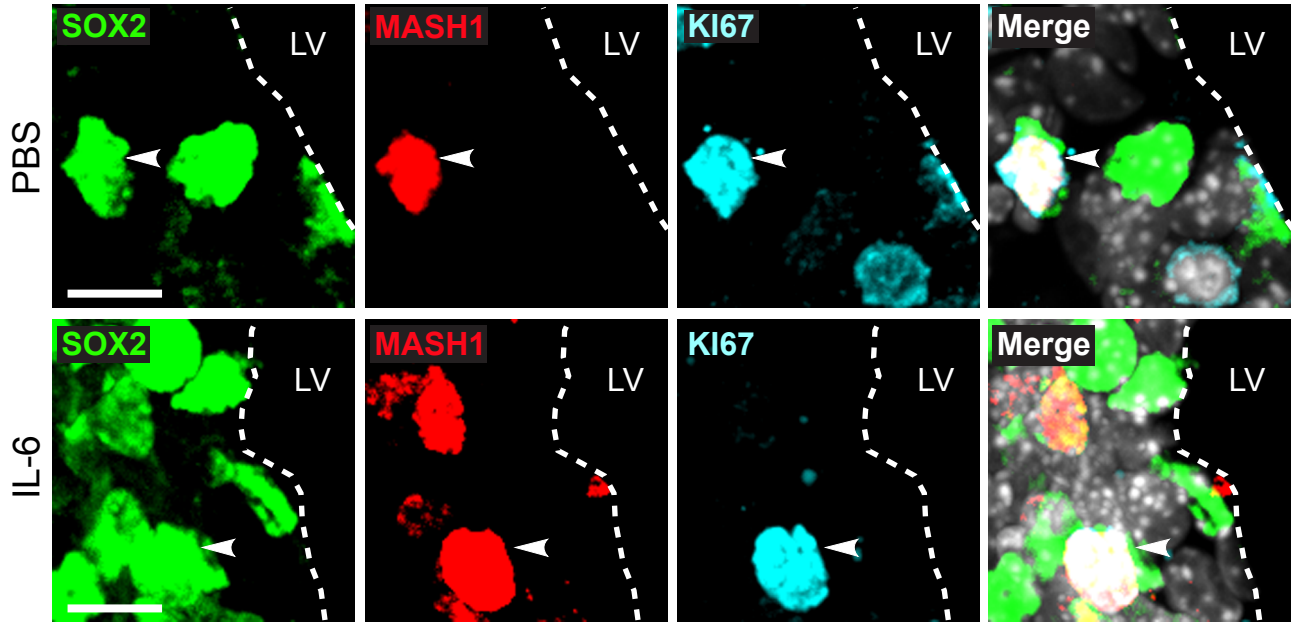

Figure S4. ***Analysis of proliferating TA cells in perinatal mice following acute exposure to a circulating surge of IL-6.*** **(Related to Figure 5).** High magnification images of coronal sections through the V-SVZ of perinatal mice injected with either IL-6 or PBS intraperitoneally on P7, injected with BrdU on P8 and immunostained on P9 for SOX2 (green), MASH1 (red) and KI67 (blue). Sections were counterstained with Hoechst 33258 (light grey in the right, merged images). The white-hatched lines indicate the borders of the lateral ventricle (LV). Arrowheads denote triple-labelled cells. Scale bars are 10 μm.

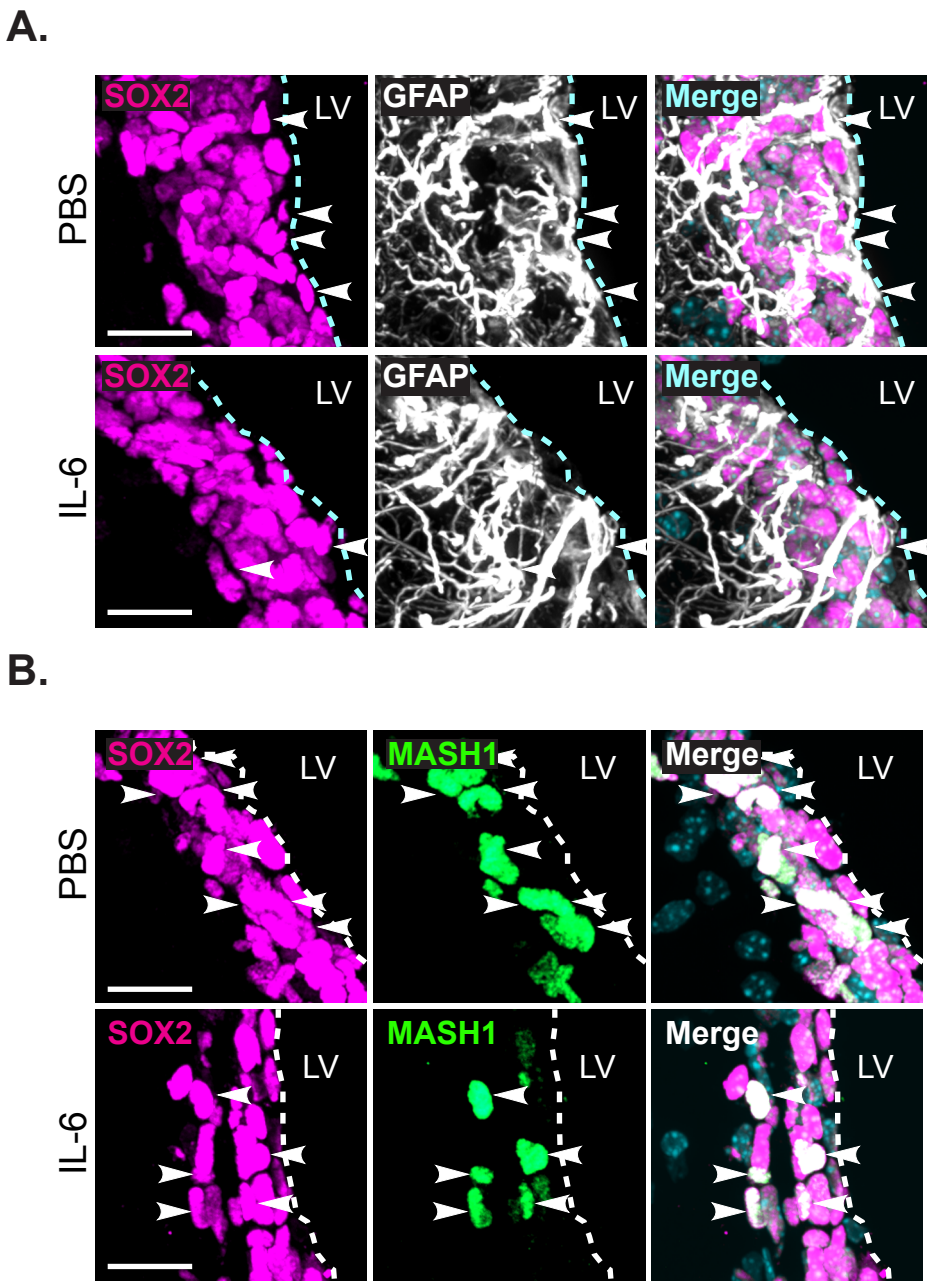

Figure S5. ***Analysis of adult V-SVZ NSCs and TA cells following exposure to a surge of circulating IL-6.*** **(Related to Figure 7).** High magnification images of coronal sections through the V-SVZ of adult mice injected with either IL-6 or PBS intraperitoneally on P42, and analyzed 15 days later on P57 by immunostaining for SOX2 (magenta) and either GFAP (white in **A**) or MASH1 (green in **B**). Sections were counterstained with Hoechst 33258 (shown in merged panels as light blue). Arrowheads denote double-labelled cells and the light blue **(A)** or white-hatched lines **(B)** indicate the borders of the lateral ventricle (LV). Scale bars are 20 μm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals and injections: For genetic ablation experiments, *IL-6r*$^{fl/fl}$ mice were crossed with *R26YFP*^{stop} reporter mice and maintained through homozygous breeding pairs on a C57/B6 background. These mice were then further crossed with *Nestin-CreERT2*$^{+/\Phi}$ mice. For perinatal ablation of IL-6R, *Nestin-CreERT2*-positive;*IL-6r*$^{fl/fl}$; *R26YFP*^{stop} males were bred with *IL-6r*$^{fl/fl}$; *R26YFP*^{stop} females.

Neurosphere cultures: For adult mice, the subependymal tissue was subjected to enzymatic digestion (1.33 mg/ml trypsin, 0.67 mg/ml hyaluronidase and 0.2 mg/ml kynurenic acid) for 40 min at 37^{0}C, and isolated in serum-free medium with trypsin inhibitor. For perinatal mice, following dissection, the subependymal tissue was mechanically dissociated into small pieces using a scalpel. Adult or perinatal tissue was then triturated into a single cell suspension using small bore-hole and fire-polished glass pipettes. Cell density and viability were determined using trypan blue exclusion. Cells were cultured under clonal conditions at 10 cells/µl in 6 well ultra-low attachment dishes (Corning) in serum-free medium containing 2% B27 supplement (Gibco), 10 ng/ml FGF (Sigma-Aldrich), 20 ng/ml EGF (Sigma-Aldrich) and 2 ug/ml heparin (Sigma-Aldrich). Cells from each animal were cultured independently, and triplicates were performed for each condition and each animal. To assay self-renewal, neurospheres were mechanically dissociated into single cell suspensions using P1000 pipette tips, passed through a 70 µm cell strainer and then cultured under the same conditions as for primary cultures, plating 2 cells/µl.

Fluorescence in situ hybridization (FISH): Freshly dissected brains of P7 or 3-month-old CD1 mice were fixed in 4% paraformaldehyde (PFA) overnight and cryopreserved in 30% sucrose for 48h. Brains were snap-frozen in O.C.T. and sectioned coronally at 16 µm. Fixed sections were washed with ethanol, followed by tissue pre-treatment, probe hybridisation and signal amplification. Sections were then immunostained with a SOX2-specific antibody (as described below) following FISH, and counter-stained with Hoechst 33258 to visualize nuclei.

Immunocytochemistry, antibodies and microscopy: For immunocytochemistry, adult mice were trans-cardially perfused with PBS, followed by 4% PFA. Following dissection, both adult and perinatal brains were fixed in 4% PFA for 24 h at 4^{0}C and cryopreserved in 30% sucrose for 48 h. Brains were then snap-frozen in O.C.T. and sectioned coronally at 18 - 20 µm. Sections were washed with PBS for 10 min and blocked using 5% BSA (Jackson ImmunoResearch) and 0.4% Triton-X-100 (Fisher) in PBS for 1 h at room temperature. Tissue sections were then incubated with appropriate primary antibodies diluted in 5% BSA in PBS, overnight at 4^{0}C. For primary antibodies raised in mouse, a MOM (mouse-on-mouse) kit was used according to the manufacturer's protocol (Vector Laboratories). Appropriate fluorescently labelled secondary antibodies (1:1000) were used for 1 h at room temperature. For visualization of nuclei, sections were counterstained with Hoechst 33258 for 5 min and slides mounted using PermaFluor mounting media (Thermo Fisher). For BrdU staining, after incubation with fluorescently labelled secondary antibodies, sections were washed 3 times with PBS, post-fixed with 4% PFA for 10 min at room temperature and washed again for 15 min with PBS. Sections were then incubated with 1M hydrochloric acid (HCl) for 10 min at 4^{0}C and 2M HCl for 10 min at room temperature and then 20 min at 37^{0}C. After extensive washes with PBS, slides were blocked with 5% normal

donkey serum, (Jackson ImmunoResearch), 0.4% Triton-X-100 and 1M glycine (BioBasics) for 1 h at room temperature. Sections were then incubated with anti-BrdU (rat, ABD Serotec) antibodies diluted in PBS (1:300), overnight at 4^0C and then with Cy5-conjugated anti-rat secondary antibodies for 1 h at room temperature. Sections were counterstained with Hoechst 33258 for 5 min and then mounted using PermaFluor mounting media (Thermo Fisher).

Analysis and statistics: For analysis of the olfactory bulbs and V-SVZ, serial coronal 18 - 20 μm sections were collected spanning the rostral-caudal extent of the olfactory bulbs and SVZ. For quantification, every 6 – 10^{th} section was selected for a total of 6 – 10 representative sections per olfactory bulb and SVZ, depending on the age of the mice (for P8/P9 mice n = 6 sections; for P21 mice n = 8 sections; for >P21 mice n = 10 sections), which were matched in terms of neuroanatomical level. Every positive cell was counted on these sections in all cases except those located within the rostral migratory stream with the exception of data quantified in Figure 4 in which positive cells within rostral migratory stream were included. To obtain the relative total number of SOX2-positive cells, or SOX2-positive cells that were also positive for MASH1 or GFAP in the SVZ, or the total number of NEUN-positive cells that were also positive for BrdU in the olfactory bulb, the total number of SOX2-positive cells or double positive cells was multiplied by the number of slides collected (for P8/P9 mice n = 6 slides; for P21 mice n = 8 slides; for >P21 mice n = 10 slides) to account for the sampling frequency. To quantify the proportion of SOX2-positive or BrdU-positive cells that were also positive for YFP, GFAP, MASH1 or KI67 within the V-SVZ, 6 – 10 sections were sampled as above and immunostained, and the total numbers of cells positive for both or a combination of three markers were counted and expressed as a percentage of double-positive/single-positive cells. In monolayer culture experiments, for proliferative index, at least 200 cells from different fields of view were counted per condition and results are representative from at least three independent experiments.