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IL-22 promotes neural stem cell self-renewal in the adult brain

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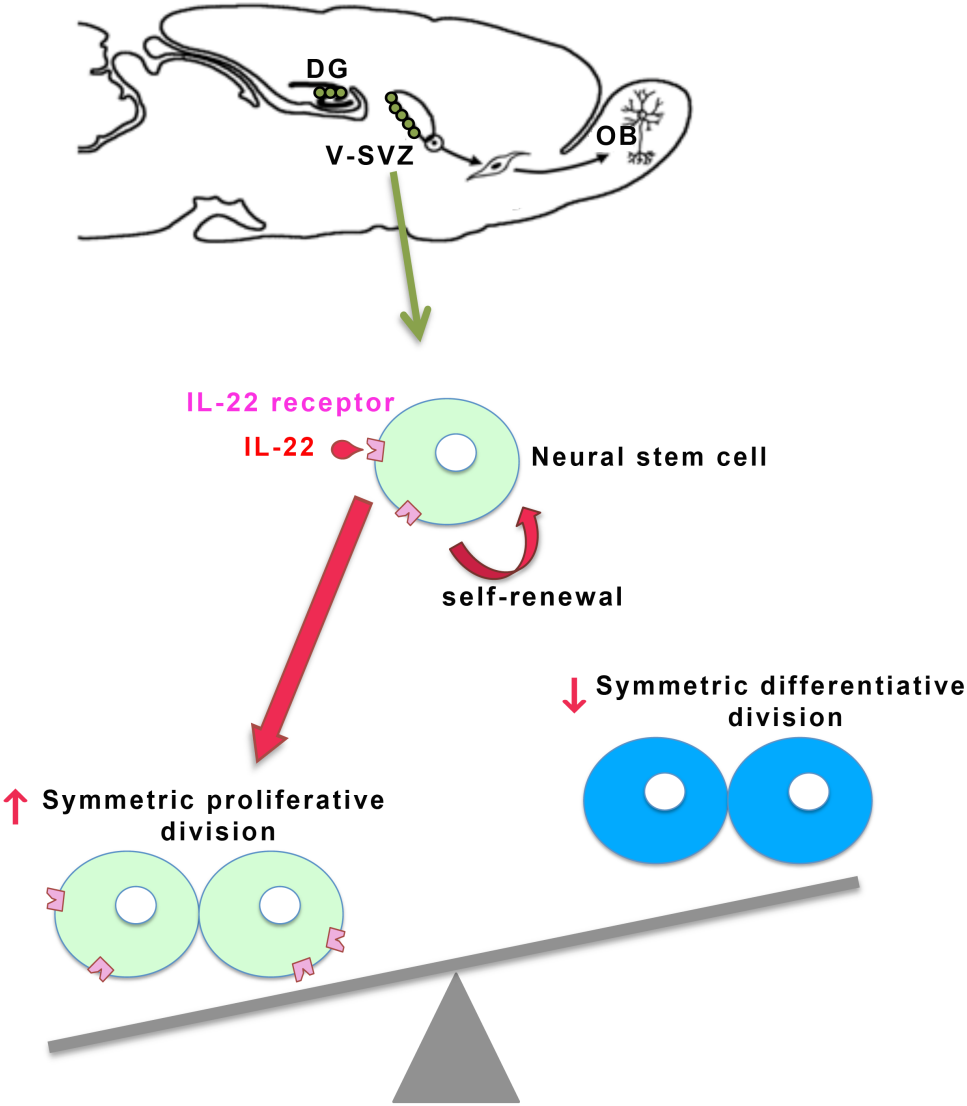
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Key words : neural stem cell, interleukin, cytokine, self-renewal, symmetric division

Abbreviation list: IL-22 : interleukin-22, NSC: neural stem cells, V-SVZ: ventricular-subventricular zone



ABSTRACT

Mainly known for its role in immune defense and inflammation, interleukin 22 (IL-22) has emerged over the past decade as a cytokine involved in the adaptation of stem/progenitor cell activity for tissue homeostasis and repair. IL-22 is present in the brain, which harbors neural stem cells (NSC) in specific niches of which the ventricular-subventricular zone (V-SVZ) is the most important. In this study, we examined a possible effect of IL-22 on NSC in the adult mouse brain. We demonstrate that the IL-22 receptor is expressed in the V-SVZ, mainly in NSC characterized by their SOX2 expression. Addition of IL-22 to V-VSZ cell cultures resulted in an increase in NSC self-renewal, associated with a shift in NSC division mode towards symmetric proliferative divisions at the expense of differentiative divisions. Conversely, loss of IL-22 in knockout mice led to a decrease in neurosphere yield, suggesting a reduction in the NSC population, which was confirmed by the decrease in cells retaining BrdU labeling in IL-22 knockout mice. Our study supports that IL-22 is involved in the development and/or maintenance of V-VSZ NSC and opens new avenues to further investigate the role of IL-22 in NSC biology in health and disease.

SIGNIFICANCE STATEMENT

Interleukin-22 (IL-22) is expressed in the healthy brain and its expression is augmented in several brain pathologies. This study demonstrates that neural stem cells (NSC) from the ventricular-subventricular zone express the IL-22 receptor and respond to IL-22 by enhancing their self-renewal through increased symmetric proliferative divisions. Loss of IL-22 in knockout mice leads to a decrease in neurosphere yield, associated with a decrease in long-term label retaining cells, confirming that IL-22 is an essential regulator of NSC activity. As IL-22 axis may be of importance in brain diseases, these results highlight the relevance of considering IL-22 for NSC recruitment in brain diseases.

INTRODUCTION

Cytokines are small-secreted proteins involved in cellular communication. Known for their role in inflammation and immune responses, cytokines are involved in a wide range of cellular functions, including cell proliferation, differentiation and survival [1,2]. Cytokines are mainly produced by immune cells but can also be synthesized by other cells types [1]. Among the cytokines expressed in the brain, interleukin 22 (IL-22) was first identified as a factor produced by activated T cells and mast cells [3]. In the healthy human brain, IL-22 is expressed by astrocytes [4]. Neuroinflammatory diseases increase IL-22 expression levels in the brain and cerebrospinal fluid [4].

Recent reports have shown that IL-22 is involved in the maintenance of tissue integrity not only by contributing to inflammation and host defenses, but also by regulating tissue homeostasis and repair [4–7]. IL-22-mediated regenerative effects are based in part on the regulation of stem cell populations. Indeed, IL-22 promotes keratinocyte stemness [8], expands hematopoietic stem cell population [9] and regenerates intestinal stem cell pool [7]. Thus, IL-22 could be a major regulator of stem cells. However, the involvement of IL-22 in the regulation of brain neural stem cells (NSC) has never been explored.

NSC persist throughout life in the adult mammalian brain within specific areas of which the ventricular-subventricular zone (V-SVZ) is the largest germinal center. NSC extend a cilium into the cerebrospinal fluid which controls NSC activity. When activated, NSC proliferate, self-renew and produce new neurons and glial cells [10]. Initially discovered in rodents, NSC have also been identified in the adult human brain [11]. NSC are recruited by brain injuries and degenerative diseases [12,13]. Importantly, ablation of newly produced cells impairs functional recovery, indicating that NSC activity is required for brain repair [14].

Given the central role of IL-22 in somatic stem cells, we investigated whether this cytokine controls adult brain NSC.

MATERIAL AND METHODS

Animals. Experiments were performed on 2 to 6-month-old mice C57BL/6J mice (Janvier Laboratories, France) and IL-22-knockout mice (IL-22KO, kindly provided by L Dumoutier, Université catholique de Louvain, Belgium). Animals were housed in the PREBIOS animal facility (University of Poitiers). Experimental procedures were carried out in accordance with the guidelines of the European Communities Council Directive and validated by the Regional Ethical Committee COMETHEA under the agreement number CE-2014-06.

Solutions and chemicals. IL-22 was purchased from R&D systems (Systems Inc., Minneapolis, MN, USA). Cell culture media and growth factors were purchased from Invitrogen (Carlsbad, CA, USA).

RT-QPCR. Total RNA was prepared from microdissected V-SVZ, hippocampus and cortex from mouse brains, or skin (positive control), reverse transcribed and subjected to real-time quantitative PCR as described previously [15] using the primer sequences provided in supplementary Table 1. Samples were normalized against housekeeping genes (GAPDH, HMBS and B2M) and reported according to the Δ CT method as relative mRNA expression: $2^{-(CT_{\text{housekeeping}} - CT_{\text{gene of interest}})}$.

Immunostaining. Immunostaining was performed as previously described [16] using the primary antibodies listed in supplementary table 2. Analyses were performed with an FV-1000 spectral confocal station installed on an IX-81 inverted microscope (Olympus).

Western blotting. Western blotting was performed as previously described [16] on secondary neurospheres derived from primary neurospheres treated or not with 20 ng.ml^{-1} IL-22, using antibodies listed in supplementary table 2. Samples were normalized against housekeeping gene actin.

Neurosphere and cell pair assays. Neurosphere and cell pair assays were performed as previously described [16], in culture medium with 0,1% BSA (control; IL-22 solvent) or with 20 ng.ml⁻¹ IL-22.

Labeling of slowly dividing V-SVZ stem cells. Two-month-old wild-type and IL-22 knockout mice were subjected to daily intraperitoneal injections of 50 mg.kg⁻¹ BrdU for 5 days. Twenty-eight days after the first injection, brains were processed for BrdU immunohistochemistry as previously described [17]. Brain sections were also processed for Ki67 immunohistochemistry.

Data analysis. Statistical significance of differences was examined by the non-parametric Mann and Whitney's test for pairwise comparison (Statview 5.00 software). The level of statistical significance was set for *p* values <0.05 and represented in the figures by: * for *p* < 0.05, ** for *p* < 0.01, **** for *p* < 0.0001.

RESULTS

IL-22 exerts its effect by binding to a heterodimeric cell surface receptor composed of the IL-10R2 and IL-22R1 subunits [18]. Analysis of IL-22 receptor expression by RT-QPCR on microdissected V-SVZ tissue showed that both IL-10R2 and IL-22R1 mRNA are expressed by V-SVZ cells (Figure 1A). Whereas the ubiquitous IL-10R2 subunit was also found, as expected, in all brain areas tested, no significant levels of IL-22R1 or IL-22 were detected by RT-QPCR in the other brain tissues analyzed (Figure 1A). No IL-22 binding protein (IL-22 BP) was detected within the V-SVZ, nor hippocampus or cortex (supplementary figure 1).

The V-SVZ contains NSC, progenitors and more differentiated cells. Therefore, IL-22 receptor immunolocalization was performed on adult mouse brain sections along with SOX2 detection to assess IL-22R1 expression specifically in NSC. Figure 1B shows SOX2-positive cells expressing the IL-22R1 receptor in the V-SVZ. To accurately identify NSC in the tissue, the V-SVZ was microdissected, dissociated into individual cells that were allowed to settle on coverslips overnight prior to immunostaining. Data analysis indicates that 7.9 ± 1.3 % of the cells in the culture express the IL-22R1 receptor (Figures 1C and 1D). Among the IL-22R1-positive cells, 75 ± 3 % are SOX2-positive, suggesting that IL-22R1 is mainly expressed by

NSC. Furthermore, of the SOX2-expressing cells, $85 \pm 3\%$ express the IL-22R1 receptor. These data support that NSC of the V-SVZ express the IL-22 receptor. In numerous cells, IL-22 recruits STAT-3 to exert its effects [5,7,19]. To determine if this pathway is also activated in V-SVZ cells, the cells were exposed to IL-22 for 30 minutes and STAT-3 phosphorylation was then examined by immunostaining. Fig 1E illustrates IL-22R1 expressing cells that are positive for PSTAT-3 following exposure to IL-22. Quantification of the data indicates that IL-22 increases the proportion of PSTAT-3 among the IL-22R1 expressing cells by 3-fold, suggesting that IL-22R1 is functionally coupled to STAT-3 signaling pathway.

IL-22 protein is present in the cerebrospinal fluid in which NSC extend a cilium [10]. We thus assessed the effects of IL-22 on the ability of NSC to produce neurospheres and self-renew. V-SVZ cells obtained from freshly microdissected mouse brain were cultured in the presence or absence of recombinant IL-22 for 7 days and the primary neurospheres produced (Figure 2A, SPHERES I) were counted. Primary neurospheres are generated by active NSC (including the slowly proliferating NSC) and thus reflect the active NSC population. They contain NSC and their progenies. To quantify the number of NSC in the primary neurospheres and thus the self-renewal process, the number of cells capable of forming new neurospheres was determined after dissociation and seeding without IL-22 (SPHERES II). The results depicted in Figure 2B show that IL-22 does not affect the proportion of primary neurospheres (SPHERES I), indicating that IL-22 does not increase the active NSC population. However, when dissociated and plated without IL-22, primary neurosphere cells that were exposed to IL-22 during primary sphere production form 50% more new neurospheres (SPHERES II), implying that IL-22 promotes NSC self-renewal. These secondary neurospheres obtained in the IL-22 condition had similar size and displayed similar multilineage potency as the secondary neurospheres obtained in control condition (Figure 2C, D and E). Additionally, we did not find any IL-22-induced gene expression in the Notch pathway that is implicated in NSC maintenance and quiescence [20] (supplementary figure 2).

The role of IL-22 in NSC self-renewal was further examined by determining the impact of IL-22 on the division pattern of NSC using a cell pair assay [16]. For this assay, V-SVZ cells isolated from mouse brains were plated with or without IL-22 in culture medium containing low levels of EGF and FGF-2 for 18 hours to allow cell pair formation (Figure 3A, supplementary figure 3). Cultures were then immunostained for the NSC marker SOX2 along with α -tubulin to accurately identify cell pairs. Three types of cell pairs were distinguished (Figure 3B): SOX2-positive pairs corresponding to symmetric proliferative divisions, SOX2-positive-SOX2-negative pairs corresponding to asymmetric divisions, and SOX2-negative

pairs corresponding to symmetric differentiative divisions. IL-22 doubled the proportion of symmetric proliferative divisions at the expense of differentiative divisions (Figure 3C), resulting in an increase in the number of NSC and most likely responsible for the IL-22-triggered increase in NSC self-renewal observed in Figure 2B.

To further investigate the effect of IL-22 on the NSC population, we analyzed the number and behavior of V-SVZ cells in IL-22-knockout mice (IL-22KO) compared to wild-type (WT) mice (Figure 4). While the total number of V-SVZ cells obtained after microdissection was not significantly different between IL-22KO (288,751 +/- 20,287) and WT mice (264,286 +/- 28,272; $p = 0.49$), the proportion of cells capable of forming neurospheres was significantly reduced in V-SVZ from IL-22KO compared to V-SVZ from WT mice, suggesting that loss of IL-22 expression reduces the stem cell pool (Figure 4A). Despite their reduced numbers, NSC from IL-22KO mice produced neurospheres that were all multipotent (Figure 4B), *i.e* containing neurons, astrocytes and oligodendrocytes, similar to NSC from wild-type mice and similar in size to neurospheres from wild-type mice ($87.2 \pm 3.7 \mu\text{m}$ for WT and $95.8 \pm 10.5 \mu\text{m}$ for IL-22KO neurospheres). Next, we traced slowly dividing V-SVZ stem cells in wild-type and IL-22KO mice *in vivo* by subjecting mice to a daily intraperitoneal injection of 50 mg.kg^{-1} BrdU for 5 days and analyzing BrdU-positive cells 24 days later within V-SVZ (Figure 4C). The number of cells retaining long-term labeling in the V-SVZ was significantly lower in IL-22KO mice (Figure 4D). Analysis of the Ki67 positive cell population showed no significant modification in the population of proliferating cells in IL-22KO mice compared to WT mice (Supplementary figure 4). Interestingly, in the hippocampus, which is another stem cell niche in the brain, loss of IL-22 in KO mice did not affect the number of cells retaining long-term labeling (Supplementary figure 5), which is consistent with the fact that there were no IL-22R1 receptors in this area (Figure 1A). Overall, these results indicate that the absence of IL-22 leads to a decrease in the number of NSC in the V-SVZ.

DISCUSSION

Our study demonstrates for the first time to our knowledge that NSC within the V-SVZ express the IL-22 receptor and that IL-22 enhances NSC self-renewal by promoting symmetric proliferative divisions at the expense of differentiative divisions, suggesting a regulatory role of IL-22 on NSC stemness.

IL-22 and its receptor have been detected in brain tissue [3] but their presence in the V-SVZ neurogenic niche had not been explored until now. Our RT-QPCR and immunohistochemical analyses showed that the IL-22 receptor is expressed in V-SVZ and that the overwhelming majority of IL-22 receptor positive cells in V-SVZ correspond to NSC. Possible IL-22 production was then examined by RT-PCR in the V-SVZ and in different brain areas. Using this method, we were unable to detect significant amounts of mRNA encoding IL-22. Although this seems discordant with the previously reported presence of IL-22 in adult brain [6], the study by Perriard *et al* detected only a small fraction of positive cells scattered throughout the tissue by immunostaining, which might be too restricted to obtain a clear signal by RT-QPCR. Nevertheless, NSC can have access to IL-22 that is present in the cerebrospinal fluid in which they extend a cilium [6,8,17], suggesting that NSC may be physiologically exposed to IL-22.

A growing body of research supports that IL-22 may play a fundamental role in tissue repair not only by acting on inflammation but also by promoting recovery through stem/progenitor cell activation [2,5–8]. Our report provides data demonstrating that, in cultures of adult brain V-SVZ cells, IL-22 increases NSC self-renewal, promoting their symmetric division. Consistent with these findings, loss of IL-22 in IL-22KO mice resulted in a decrease in the number of stem cells in the V-SVZ, as determined by their ability to form neurospheres as well as their long-term label retention properties. This effect of IL-22 appeared to be limited to the V-SVZ neurogenic niche as we were unable to detect IL-22R in the hippocampus and there was no change in the number of long-term label-retaining stem cells *in vivo* within the hippocampus of IL-22KO mice. Such differential cell-origin-dependent effects on V-SVZ versus hippocampal NSC have been already described for inflammation [21]. Taken together, these data suggest that IL-22 is a master regulator of physiological stemness.

This effect of IL-22 on NSC may be of interest in brain pathologies. Among these, multiple sclerosis is a demyelinating pathology in which patients have increased levels of IL-22 in serum and cerebrospinal fluid [4,22,23]. Interestingly, in IL-22 binding protein (IL-22BP) knockout mice, the greater availability of IL-22 is associated with an overall less severe disease course compared to wild-type littermates, suggesting a protective role for IL-22 [24]. Such a beneficial effect of IL-22 has been shown in mice after ischemia [19]. Because V-SVZ NSC/progenitors are implicated in recovery from stroke [25] and recruited in multiple sclerosis [13], our study showing an effect of IL-22 on NSC suggests that IL-22 may

implicate NSC in improving recovery from brain diseases, a hypothesis that nevertheless remains to be formally tested.

CONCLUSION

Our study demonstrates that IL-22 controls the NSC population in the adult brain. Because NSC are recruited in brain pathologies and taking into account that modified IL-22 molecules have been generated with potential candidates already in clinical trials [26], the role of IL-22 on NSCs that we describe in this study may be of interest in the context of brain repair.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate that they have no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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FIGURES

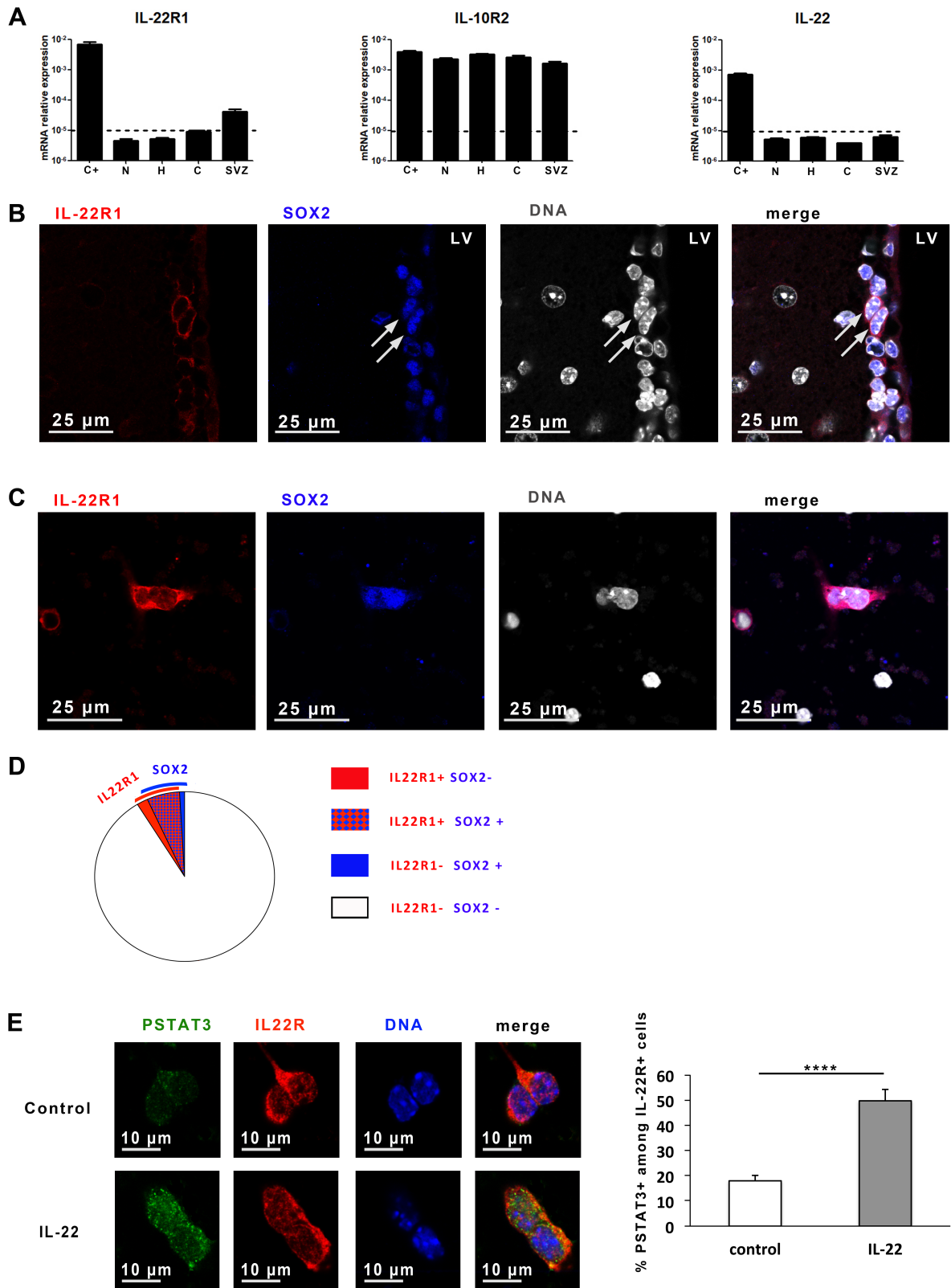


Figure 1: Expression of IL-22 receptor in NSC and coupling to STAT-3 signaling pathway

A. Relative mRNA expression to housekeeping genes for IL-22, IL-22R1 and IL-10R2 in neurosphere (N), hippocampus (H), cortex (C), V-SVZ (SVZ) and in an inflammatory skin sample as a positive control (C+) was determined by RT-QPCR analysis. Data are expressed as means \pm s.e.m. of 5 independent experiments. **B.** Immunodetection of IL-22R1 (red) with the nuclear stem cell marker SOX2 (blue) in mouse brain sections. The nuclei (DNA) of all cells were stained with DAPI (white). LV: lateral ventricles. Arrows indicate cells within the V-SVZ that express both IL-22R1 and SOX2. **C.** Immunostaining of IL-22R1 (red) along with the nuclear stem cell marker SOX2 (blue) on dissociated V-SVZ cells plated on polylysine-coated coverslips for 18 hours in culture medium containing low concentrations of EGF and FGF2. The nuclei (DNA) of all cells were stained with DAPI (white). **D.** Quantification of different cell subpopulations: IL-22R1-positive/ SOX2-negative (red), IL-22R1-positive/ SOX2-positive (red and blue), IL-22R1-negative/ SOX2-positive (blue) or IL-22R1-negative/ SOX2-negative (white). Data are based on counting a total of 5880 cells. **E.** Immunostaining of IL-22R1 (red) along with PSTAT-3 (green) on dissociated V-SVZ cells exposed during 30 minutes to 20 ng.ml⁻¹ IL-22 (IL-22) or to control solution (Control). The nuclei (DNA) of all cells were stained with DAPI (blue). Percentage of PSTAT-3 positive cells among IL-22R1 positive cells in the control and IL-22 conditions. More than 200 IL-22R1 positive cells were counted for each condition within each experiment. Data represent means \pm s.e.m of 3 independent experiments, with each condition assessed in duplicate per experiment. ****: $p < 0.0001$.

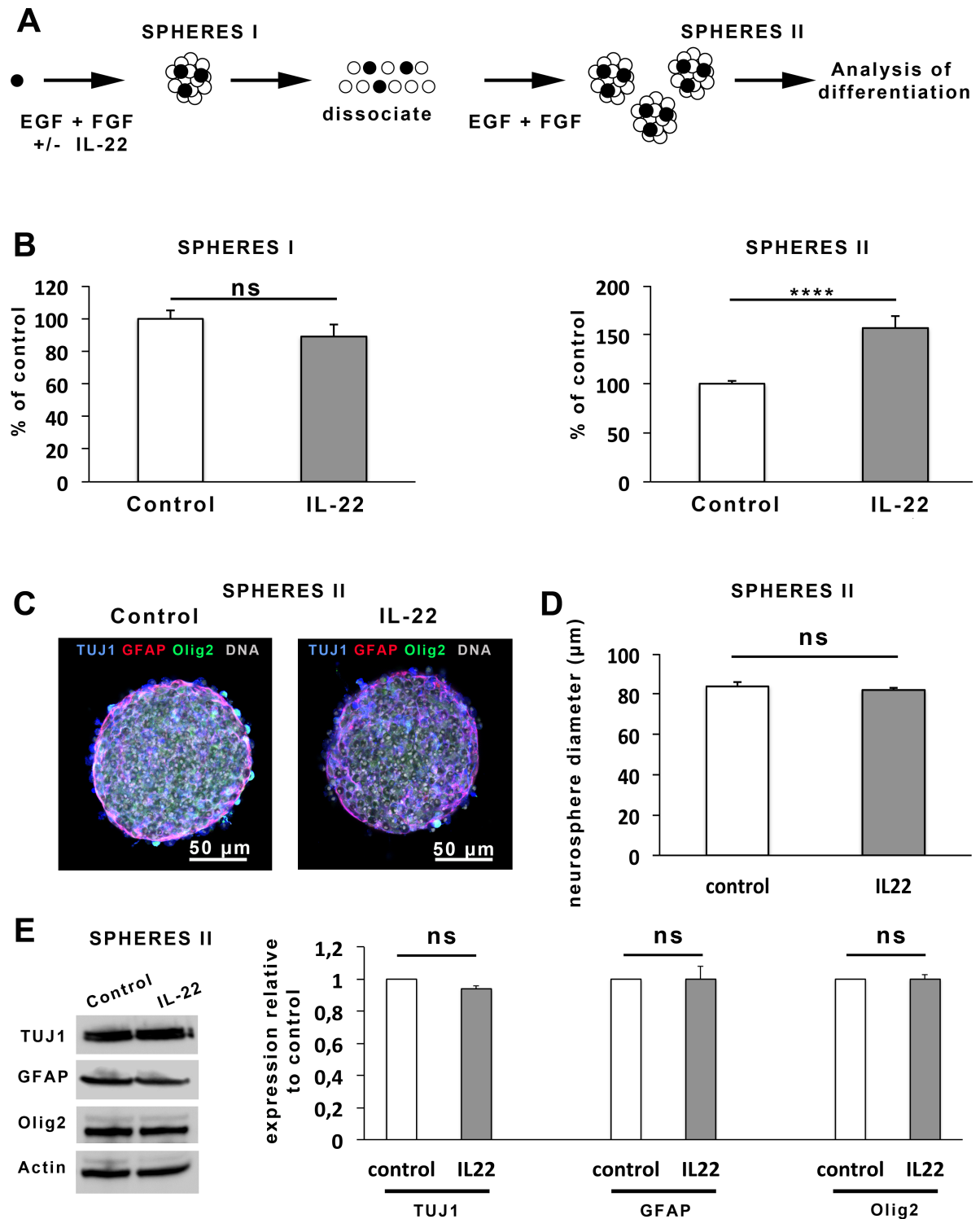


Figure 2: IL-22 promotes NSC self-renewal

A. Schematic of the neurosphere assay. V-SVZ were dissected from the brain, dissociated into individual cells, and plated in their culture medium with or without 20 ng.ml⁻¹ IL-22. Of the plated cells, active NSC (black circles) produce neurospheres (SPHERES I) following one week *in vitro*. These neurospheres contain NSC and their progenies (white circles). To determine the number of NSC in spheres I, the spheres were collected, dissociated as single cells and plated with EGF and FGF-2. After one week, neurospheres (SPHERES II) derived from NSC were obtained. These secondary spheres (SPHERES II) were allowed to differentiate for the analysis of their capacity to produce neurons, astrocytes and

oligodendrocytes. **B.** Graphical representation of the number of primary neurospheres (SPHERES I) obtained after one week of culture with either 20 ng.ml⁻¹ IL-22 or control medium and the number of secondary neurospheres (SPHERES II) obtained following one week of culture of V-SVZ cells derived from primary spheres. Data are expressed as percentage of control and represent means \pm s.e.m of 6 independent experiments, with each condition assessed in triplicate per experiment. ns: not significant, ****: $p < 0.0001$. **C.** Micrographs of secondary neurospheres (SPHERES II) derived from primary neurospheres that had been exposed to control or IL-22 condition. The neurospheres (SPHERES II) were allowed to differentiate for one week. The presence of neurons (TUJ1, blue), astrocytes (GFAP, red) and oligodendrocytes (Olig2, green) was assessed by immunostaining. Nuclei (DNA) were stained with DAPI (white). **D.** Diameters of the secondary neurospheres (SPHERES II) derived from primary neurospheres that had been exposed to control or IL-22 condition. Data represent means \pm s.e.m of 3 independent experiments (n= 426 neurospheres in control condition, n = 492 neurospheres in IL-22 condition). ns: not significant. **E.** Western blot and quantification of TUJ1, GFAP and Olig2 expression in SPHERES II derived from primary neurospheres that had been exposed to control or IL-22 condition. Data represent means \pm s.e.m of 4 independent experiments, ns, no statistically significant difference.

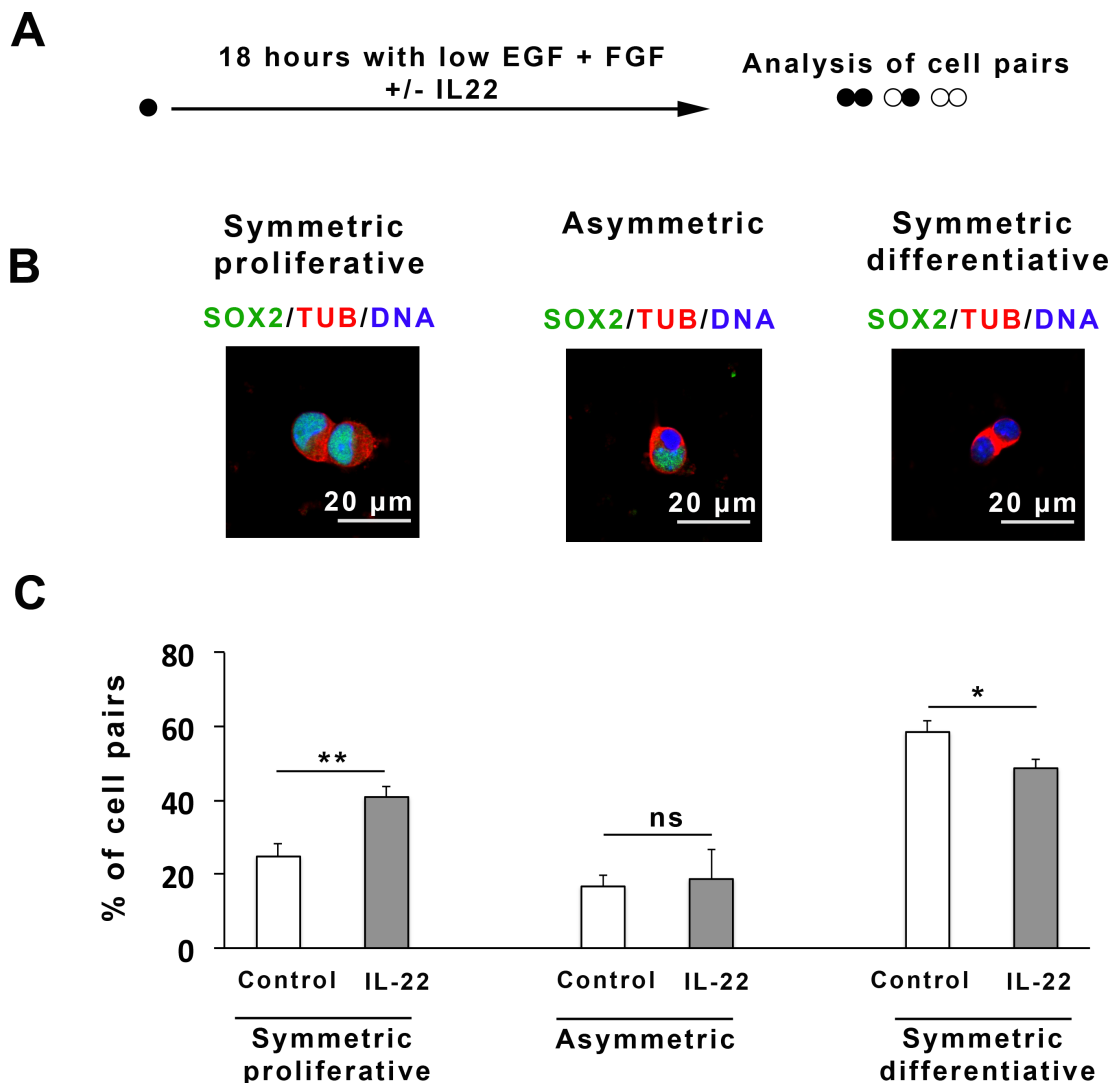


Figure 3: IL-22 increases symmetric proliferative divisions

A. Schematic of the cell pair assay. V- SVZ were dissected from the brain, dissociated into individual cells and plated in their culture medium with or without 20 ng.ml⁻¹ IL-22 for 18 hours to allow for cell pair formation. NSC are represented by black circles and progenitors by white circles. **B.** Cell pairs were then analyzed after immunostaining for SOX2 (in green), α -tubulin (TUB, red), nuclei (DNA, blue). The separate colors for each staining are provided in supplementary figure 3. **C.** Percentages of symmetric proliferative (SOX2 positive/SOX2-positive), asymmetric (SOX2-positive/SOX2-negative) or symmetric differentiative (SOX2-negative/SOX2-negative) cell pairs in cultures maintained for 18 h without (control) or with 20 ng.ml⁻¹ IL-22. Data are expressed as percentage of all cell pairs counted and represent the means \pm s.e.m of 7 independent cultures for a total number of 743 cell pairs analyzed. ns: not significant, *: p=0.048, **: p=0.0077

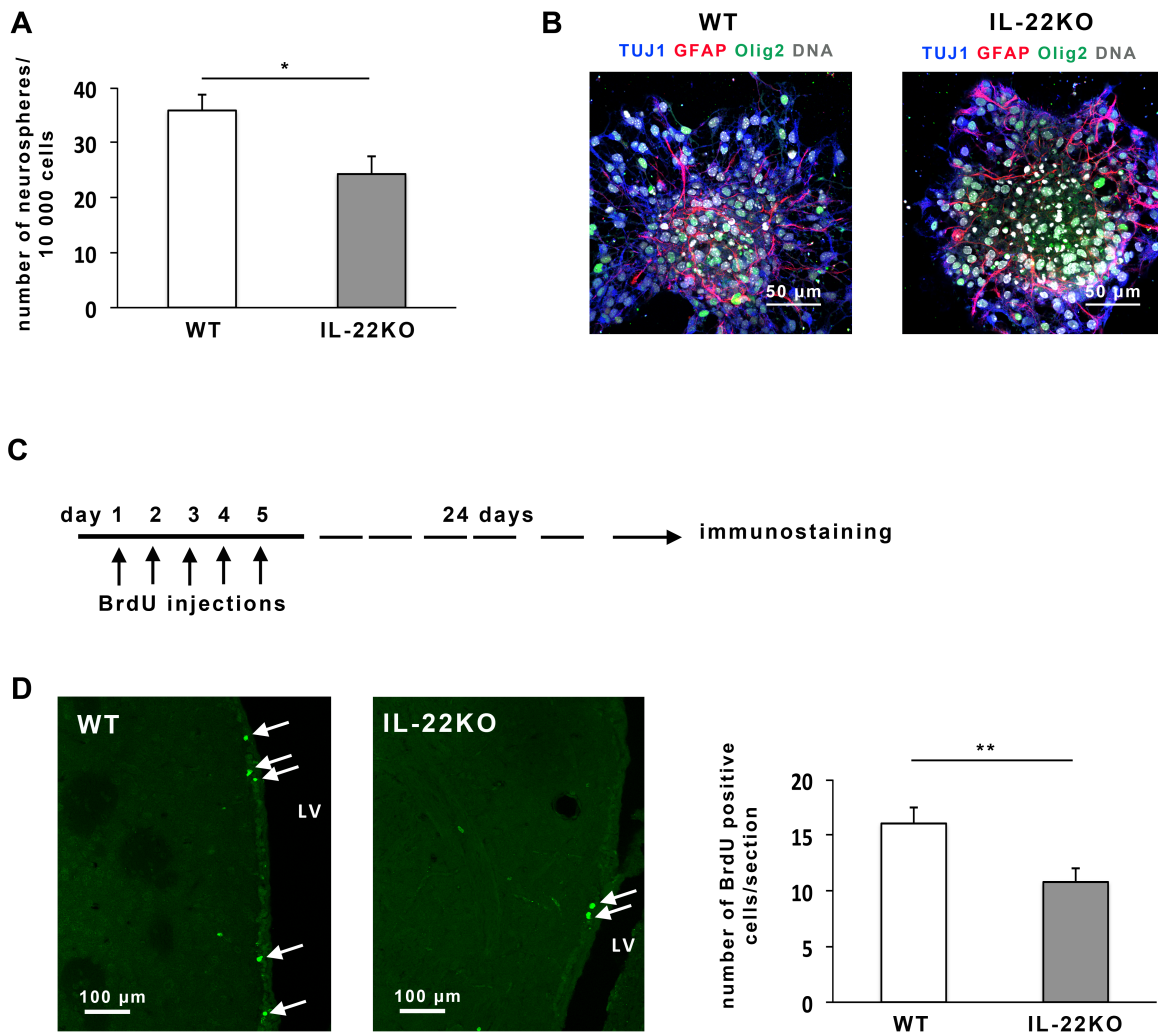
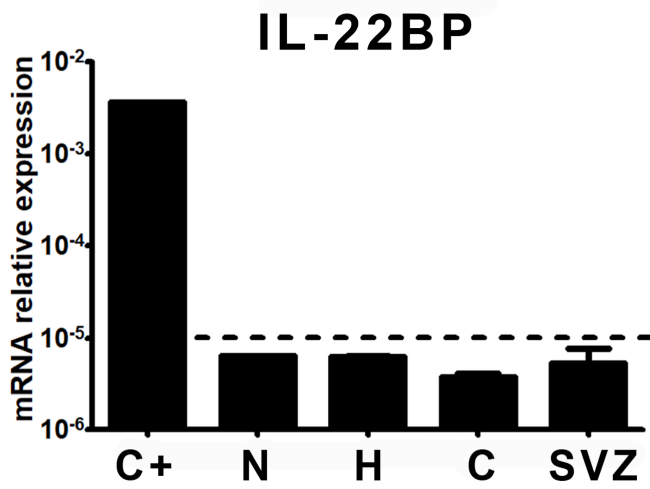


Figure 4: V-SVZ analysis of wild-type and IL-22 knockout mice.

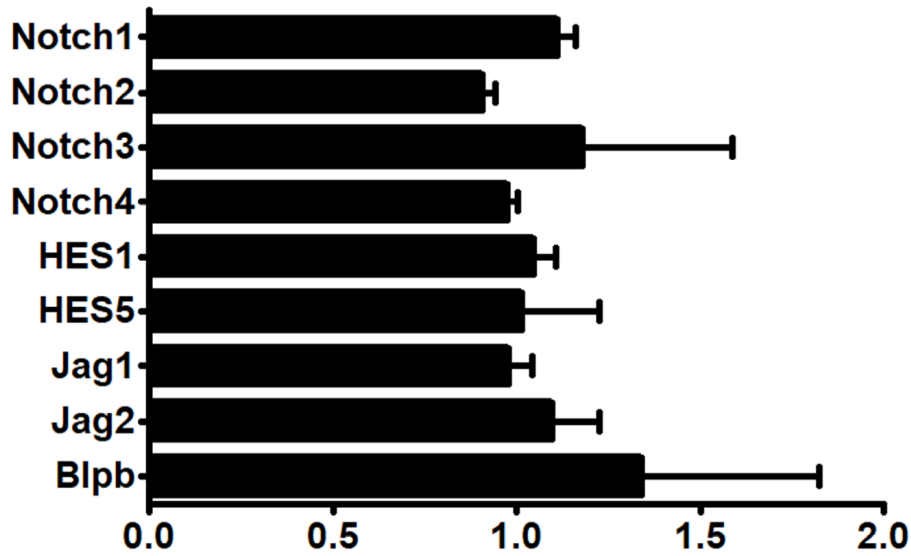
A. Number of neurospheres obtained from 10 000 V-SVZ cells of wild-type (WT, white bar) and IL-22 knockout mice (IL-22KO, grey bar). Data represent means \pm s.e.m of 11 wild-type and 11 IL-22KO mice. *: $p = 0.015$. **B.** Micrographs of neurospheres derived from wild-type and IL-22KO mice. Neurospheres were allowed to differentiate for one week. The presence of neurons (TUJ1, blue), astrocytes (GFAP, red) and oligodendrocytes (Olig2, green) was assessed by immunostaining. Nuclei (DNA) were stained with DAPI (white). **C.** Schematic of the assay used for detection of label-retaining stem cells. **D.** The number of slowly dividing V-SVZ stem-like cells in wild-type (WT, white bar) or IL-22 knockout (IL22KO, gray bar) mice was obtained by subjecting mice to daily intraperitoneal injections of 50 mg.kg^{-1} BrdU for 5 days and analyzing BrdU-positive cells in the V-SVZ 24 days later. The micrographs on the left show examples of BrdU labeling (green) in wild-type (WT) and in IL-22 knockout (IL-22KO) mice. The white arrows point BrdU positive cells. LV: lateral ventricle. For each mouse, BrdU positive cells were counted on 10 regularly spaced $40 \mu\text{m}$ -thick brain sections sampled at similar anatomical levels between 1.42 anterior to Bregma and 0.2 posterior to Bregma. Data represent means \pm s.e.m of BrdU positive cells in the SVZ per brain section. These data were obtained from counts on 9 wild-type and 14 IL-22KO mice. **: $p = 0.0068$.

Supplementary data



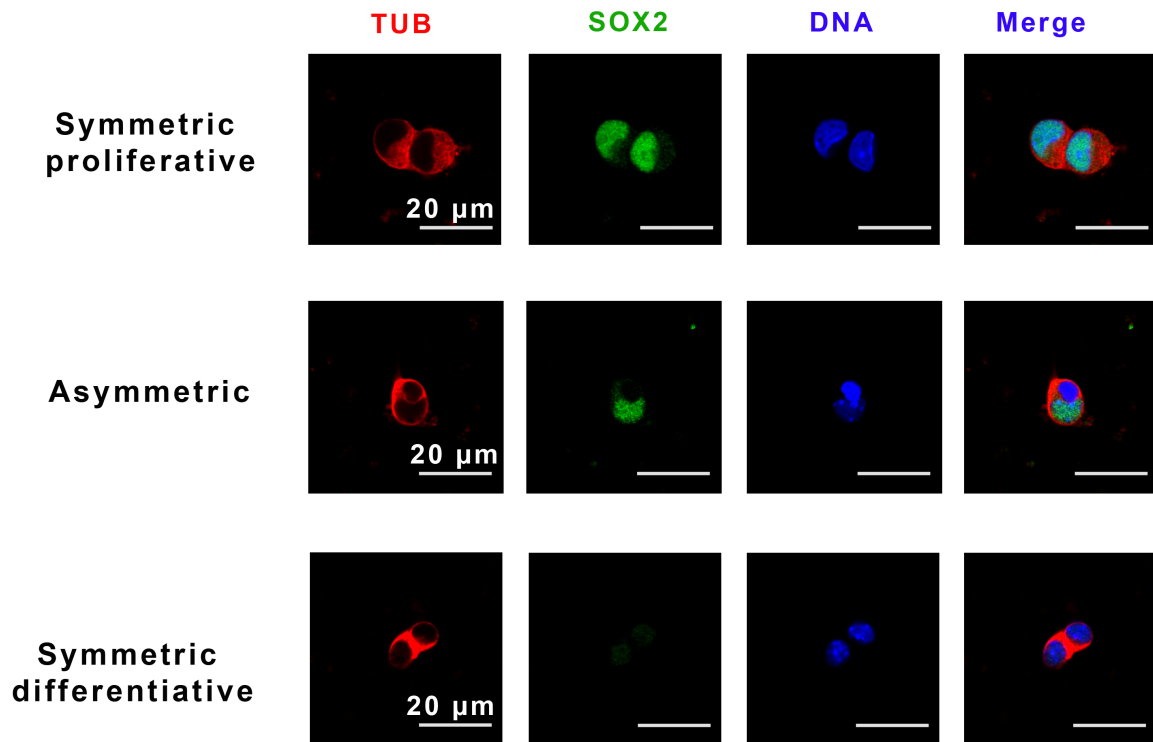
Supplementary Figure 1: Relative mRNA expression to housekeeping genes for IL-22BP in neurosphere (N), hippocampus (H), cortex (C), V-SVZ (SVZ) and in an inflammatory skin sample as a positive control (C+) was determined by RT-QPCR analysis. Data are expressed as means \pm s.e.m. of 5 independent experiments.

mRNA fold increase (IL-22/control)



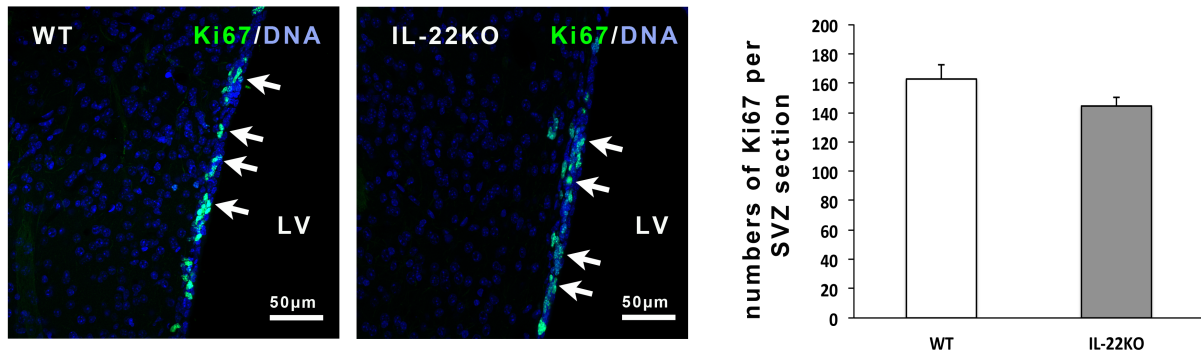
Supplementary figure 2: Effect of IL-22 on Notch signaling

Freshly dissociated V-SVZ cells were maintained during 24 hours in their culture medium in the absence (control) or presence of 20 ng.mL⁻¹ of IL-22. The cells were then collected and subjected to RT-QPCR analysis to detect Notch signaling transcripts. The data represent the fold of increase of mRNA expression in the presence of IL-22 over control. Data are expressed as means \pm s.e.m. of 8 mice (4 mice for control condition, 4 mice for IL-22 condition). There was no significant increase of Notch-related transcripts in IL-22 treated V-SVZ cells.



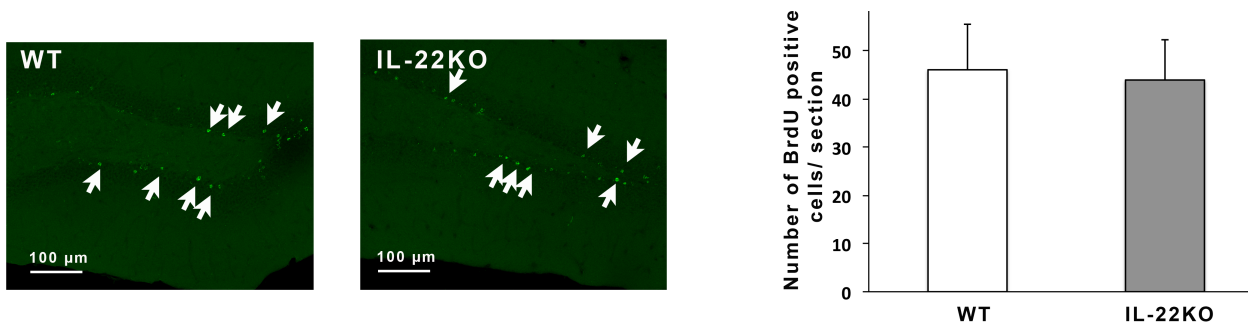
Supplementary figure 3: Separate color channels from the micrographs of Figure 3B

Immunostaining of cell pairs for α -tubulin (TUB, red), SOX2 (in green), nuclei (DNA, blue). The merge is on the right and is shown in figure 3B.



Supplementary figure 4: Analysis of Ki67 expressing cells in the SVZ of WT and IL-22KO mice

The micrographs on the left show examples of Ki67 labeling (green) in wild-type (WT) and in IL-22 knockout (IL-22KO) mice. Nuclei were labeled with TOPRO-3 (blue). The white arrows point Ki67 positive cells. LV: lateral ventricle. For each mouse, Ki67 positive cells were counted on 4 regularly spaced 40 μm -thick brain sections sampled at similar anatomical levels between and 1.2 anterior to Bregma to 0.2 posterior to Bregma. Data represent means \pm s.e.m of Ki67 positive cells in the SVZ per brain section. These data were obtained from counts on 7 wild-type and 8 IL-22KO mice. The difference was not statistically significant ($p=0.13$).



Supplementary Figure 5: The number of slowly dividing stem cells in the hippocampus of wild-type and IL-22 knockout mice was obtained by subjecting mice to daily intraperitoneal injections of 50 $\text{mg}\cdot\text{kg}^{-1}$ BrdU for 5 days and analyzing BrdU-positive cells in the hippocampus 24 days later. The micrographs on the left show examples of BrdU labeling (green) in wild-type (WT) and in IL-22 knockout (IL-22KO) mice. The arrows point some BrdU positive nuclei.

For each mouse, BrdU positive cells were counted on 10 regularly spaced 40 μm -thick brain sections sampled at similar anatomical levels between -1,06 and -2,54 anterior to Bregma. Data represent means \pm s.e.m of BrdU positive cells per brain section. These data were obtained from counts on 9 wild-type and 14 IL-22KO mice. There is no significant difference between wild-type and IL-22-KO mice.

Supplementary table 1. Primer sequences used for RT-QPCR

Genes	Primer forward	Primer Reverse
<i>GAPDH</i>	TGCGACTTCAACAGCAACTC	CTTGCTCAGTGTCTTGCTG
<i>B2M</i>	TGGTGCTTGTCTCACTGACC	CCGTTCTTCAGCATTGGAT
<i>HMBS</i>	CAGAGAAAGTTCCCCACCT	CAGGACGATGGCACTGAAT
<i>IL-22</i>	ACATGCAGGAGGTGGTACCTTT	ATCGCCTTGATCTCTCCACTCT
<i>IL-22R1</i>	ACATCACCAAGCCACCTGTA	GGTCCAAGACAGGGATCAGT
<i>IL-10R2</i>	TGGAGTGAACCCATCTGTGAAC	CCACCAGGACGGAGACTATGA
<i>IL-22BP</i>	CTCTCTCCCAGCAACAACAG	GGTCCCCCAGCAGTCAACTT
<i>Notch1</i>	ATGTCAATGTTTCGAGGACCAG	TCACTGTTGCCTGTCTCAAG
<i>Notch2</i>	GTGGCAGAGTTGATCAATTG	ATATCTCTGTTGGCCCCATTC
<i>Notch3</i>	GGGTCCTGAGGTGATCGGCT	AGGGTGCTGTGTTCTCGC
<i>Notch4</i>	ATCACAGGATGACTGGCCTC	ACTCGTACGTGTCGCTTCCT
<i>Hes11</i>	CTACCCCAGCCAGTGTCAAC	ATGCCGGGAGCTATCTTTCT
<i>Hes5</i>	GGGGTTGTTTTGTGTTTGCA	GGCCCTGAAGAAAGTCCTCT
<i>Jag1</i>	GGGAACCCTGTCAAGGAAAT	GAGCTCAGCAGAGGAACCAG
<i>Jag2</i>	GGCAAAGAATGCAAAGAAGC	GCTCAGCATTGATGCAGGTA
<i>Blbp</i>	GGAAGCTGACAGACAGTCAGA	CGCCCAGAGCTTTCATGTAC

Supplementary table 2. Antibodies used for immunostaining and Western blotting

Antigen	Source	Dilution used for immunostaining	Dilution used for Western blotting
IL-22R1	monoclonal rat (R&D Systems, ref MAB42941)	20 $\mu\text{g.ml}^{-1}$	
SOX2	polyclonal goat (Santacruz, ref: sc-17320)	1/200	
α Tubulin	monoclonal mouse, (Sigma, ref: T6199)	1/500	
GFAP	polyclonal chicken (Abcam, ref: ab4674)	1/3000	
Olig2	polyclonal rabbit (Santacruz, ref: sc-48817)	1/100	1/1000
β III Tubulin (TuJ1)	monoclonal mouse (Sigma Aldrich, ref: T8660)	1/200	
PSTAT-3	polyclonal rabbit (Cell Signalling, ref : 9145S)	1/200	
Actin	polyclonal rabbit (Sigma, ref : A2066)		1/1000
GFAP	polyclonal rabbit (DAKO, ref : 20334)		1/1000
β III Tubulin (TuJ1)	polyclonal rabbit (Abcam, ref : ab52623)		1/1000
Ki67	monoclonal rabbit (Abcam, ref: ab16667)	1/200	