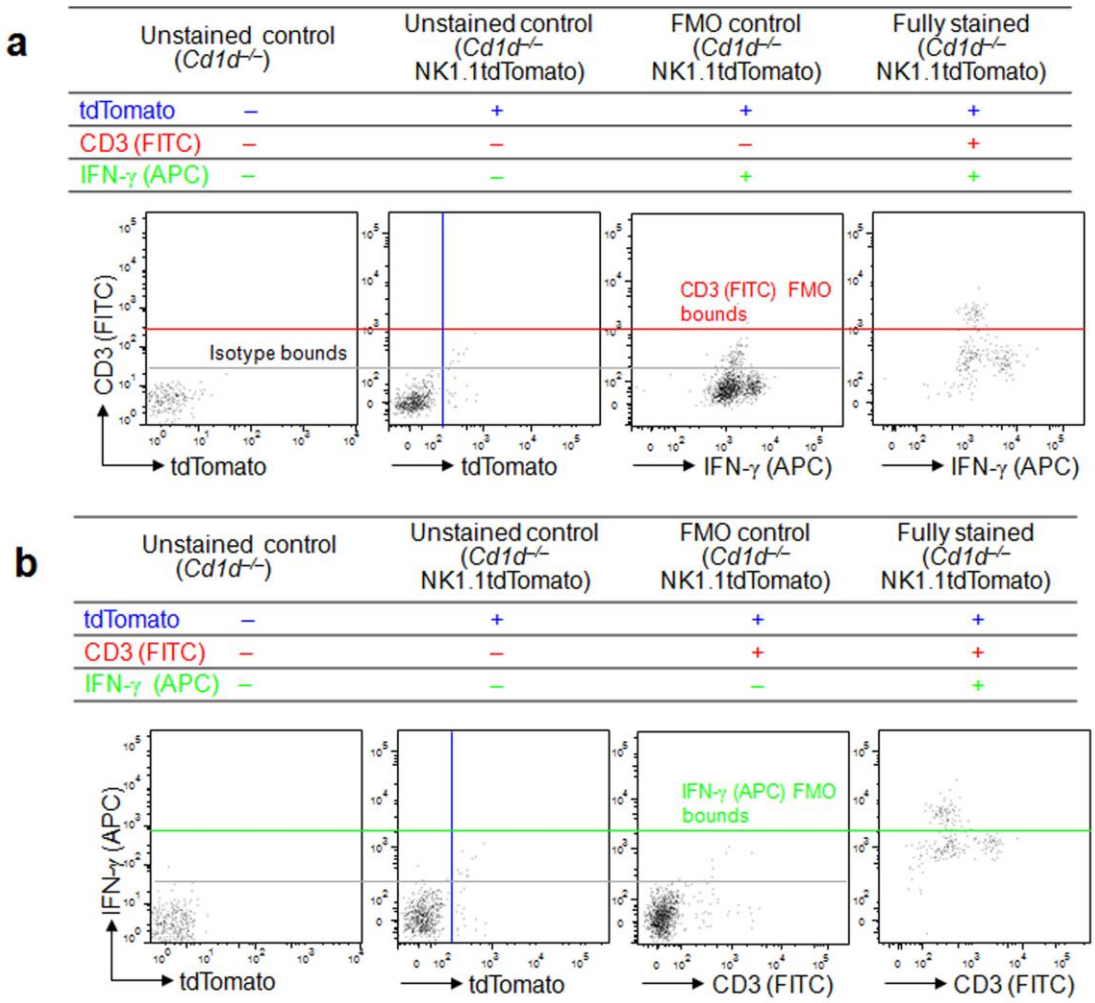
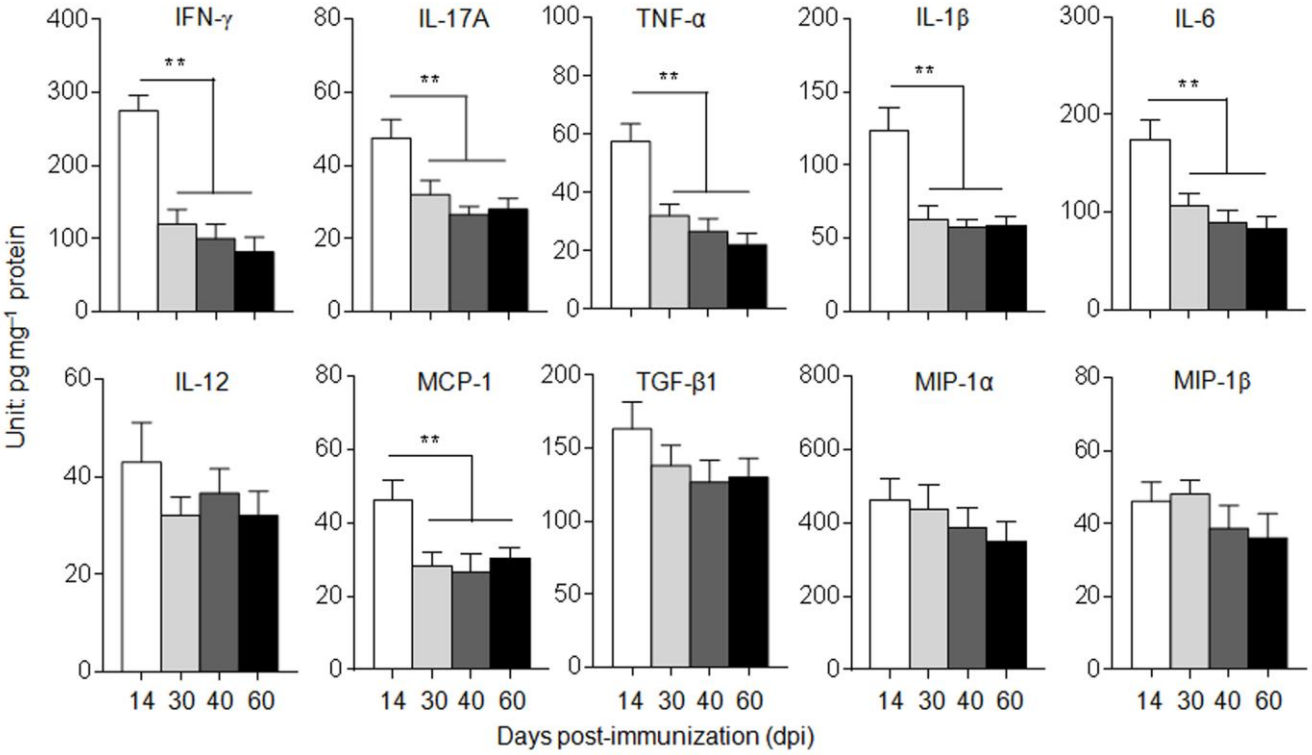


Supplementary Figure 1

Fluorescence minus one (FMO) controls for the identification of CNS-infiltrating NK cells.

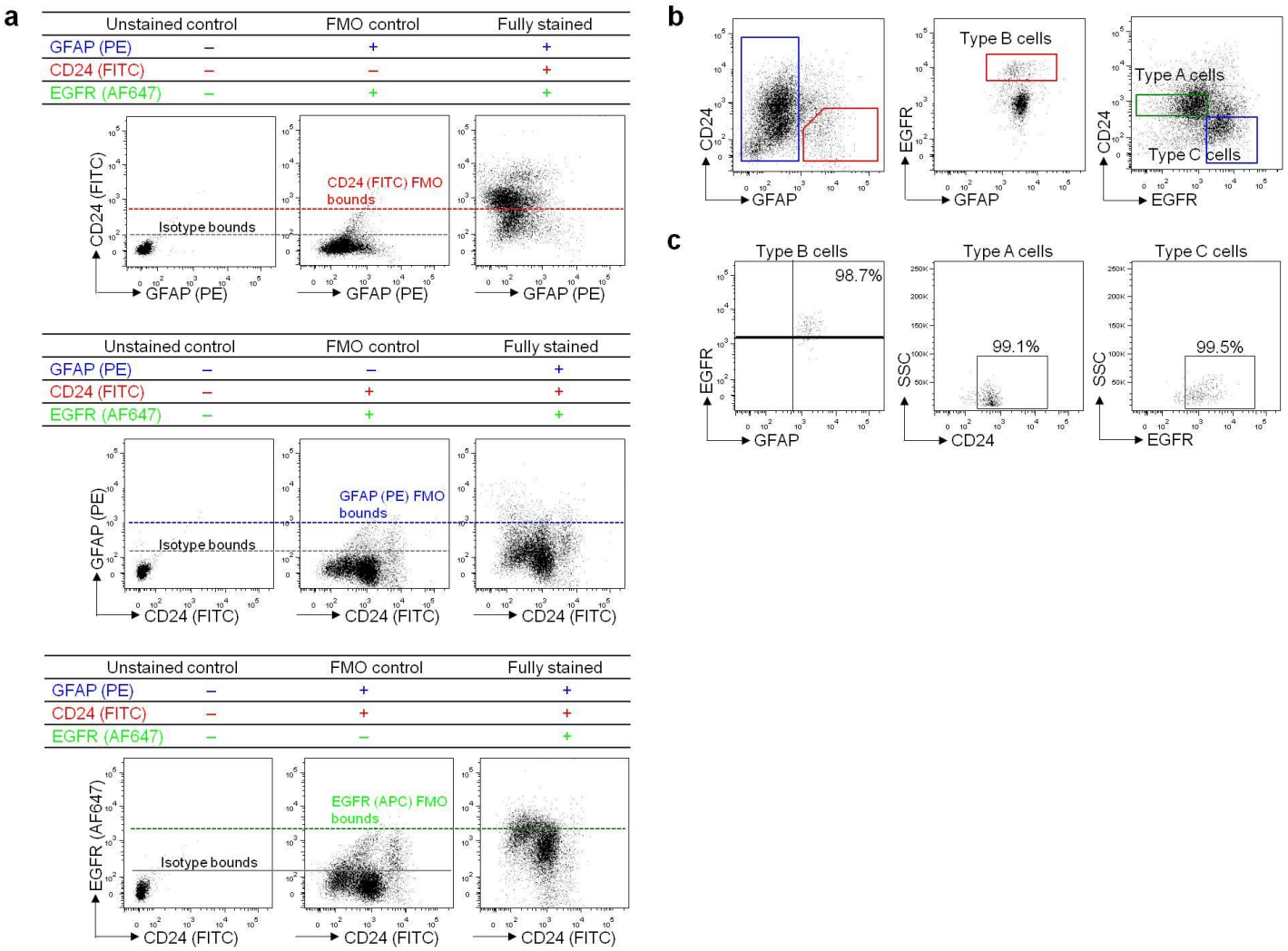
Single cell suspensions were prepared from CNS tissues of *Cd1d*$^{-/-}$ or *Cd1d*$^{-/-}$NK1.1-tdTomato^{+} mice at day 30 of EAE and stained with fluorochrome-conjugated antibodies. **a-b.** Representative flow cytometry dot plots show the identification of gating boundaries of CD3 (FITC), td-Tomato and IFN-γ (APC) with corresponding FMO controls.



Supplementary Figure 2

Reduced expression of proinflammatory factors during the recovery phase of EAE.

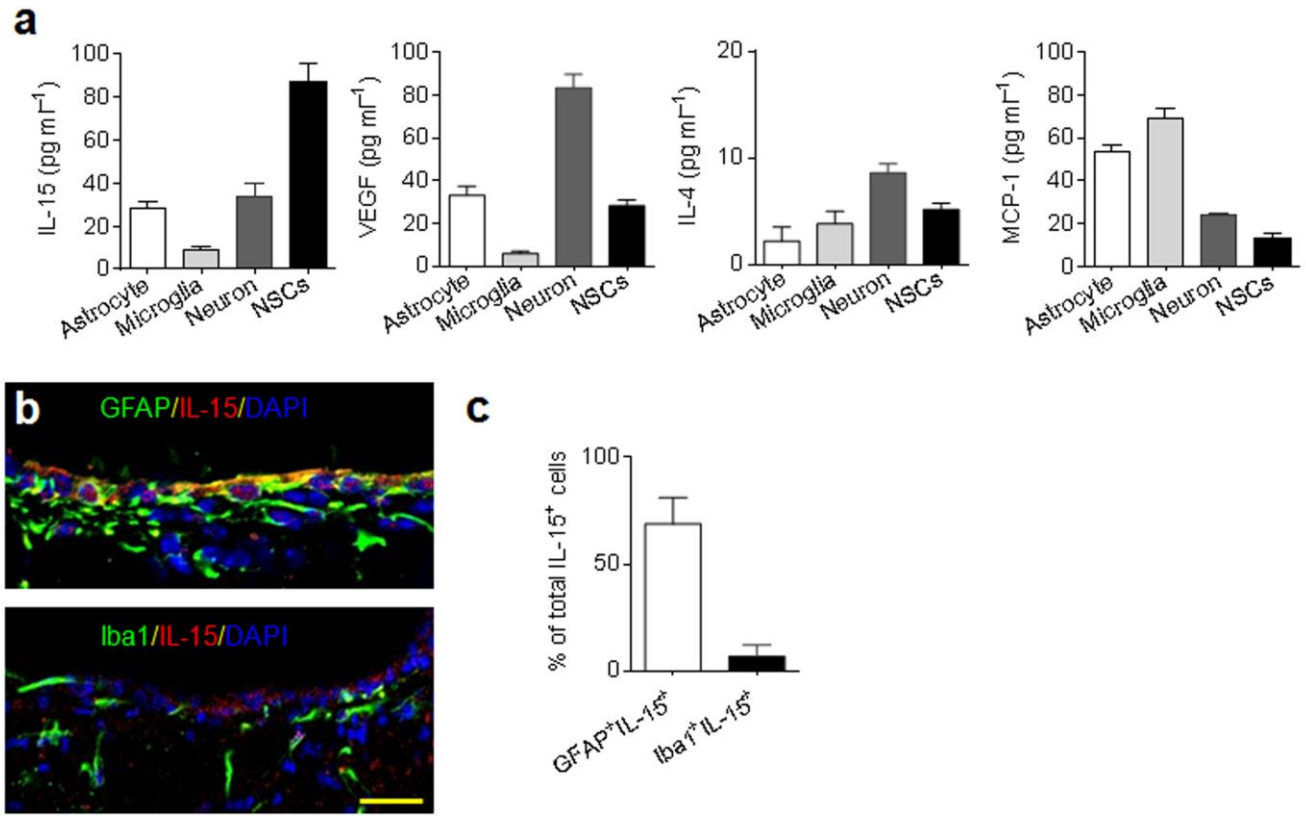
CNS homogenates were prepared from EAE mice of the indicated groups at 14, 30, 40, 60 dpi. Concentrations were measured by a Multi-Analyte ELISArray kit. Average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 3.5 ± 0.7 at 14 dpi, 2.5 ± 0.7 at 30 dpi, 2.3 ± 0.8 at 40 dpi and 2.1 ± 0.8 at 60 dpi. n = 9 per group. IFN-γ: $P < 0.001$ (14 dpi versus 30 dpi), $P < 0.001$ (14 dpi versus 40 dpi), $P < 0.001$ (14 dpi versus 60 dpi). F (3, 32) = 8.30, one-way ANOVA. IL-17A: $P < 0.001$ (14 dpi versus 30 dpi), $P < 0.001$ (14 dpi versus 40 dpi), $P < 0.001$ (14 dpi versus 60 dpi). F (3, 32) = 6.11, one-way ANOVA. TNF-α: $P < 0.001$ (14 dpi versus 30 dpi), $P < 0.001$ (14 dpi versus 40 dpi), $P < 0.001$ (14 dpi versus 60 dpi). F (3, 32) = 6.83, one-way ANOVA. IL-1β: $P < 0.001$ (14 dpi versus 30 dpi), $P < 0.001$ (14 dpi versus 40 dpi), $P < 0.001$ (14 dpi versus 60 dpi). F (3, 32) = 7.16, one-way ANOVA. IL-6: $P < 0.001$ (14 dpi versus 30 dpi), $P < 0.001$ (14 dpi versus 40 dpi), $P < 0.001$ (14 dpi versus 60 dpi). F (3, 32) = 5.54, one-way ANOVA. MCP-1: $P < 0.001$ (14 dpi versus 30 dpi), $P < 0.001$ (14 dpi versus 40 dpi), $P < 0.001$ (14 dpi versus 60 dpi). F (3, 32) = 3.39, one-way ANOVA. Results are from three individual experiments. Error bars represent s.e.m.; ** $P < 0.01$.



Supplementary Figure 3

FMO controls for the identification of SVZ type B, type C and type A cells.

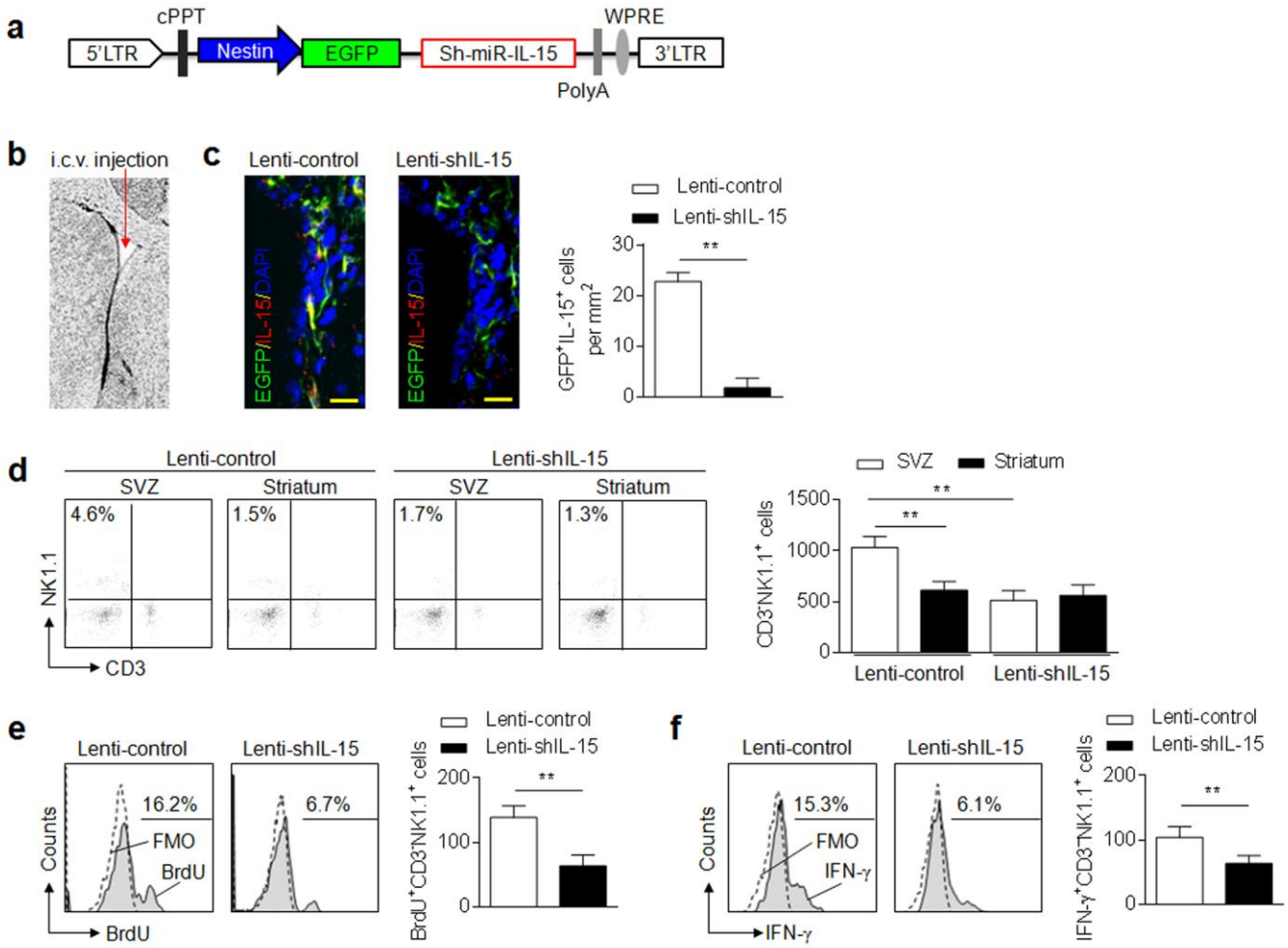
Single cell suspensions were prepared from dissociated SVZ tissues of WT adult mice and stained with fluorochrome-conjugated antibodies. **a.** Representative flow cytometry dot plots show the identification of gating boundaries of GFAP (PE), CD24 (FITC) and EGFR (AF647) with corresponding FMO controls. **b.** Representative flow cytometry dot plots show SVZ type B ($GFAP^{+}EGFR^{+}CD24^{-}$), type C ($GFAP^{-}EGFR^{+}CD24^{-}$) and type A ($GFAP^{-}EGFR^{-}CD24^{low}$) cell types from WT mice before sorting. **c.** Representative flow cytometry dot plots show purity of SVZ type B, type C and type A cell types (>98%) after sorting.



Supplementary Figure 4

Representative factors produced by CNS cells.

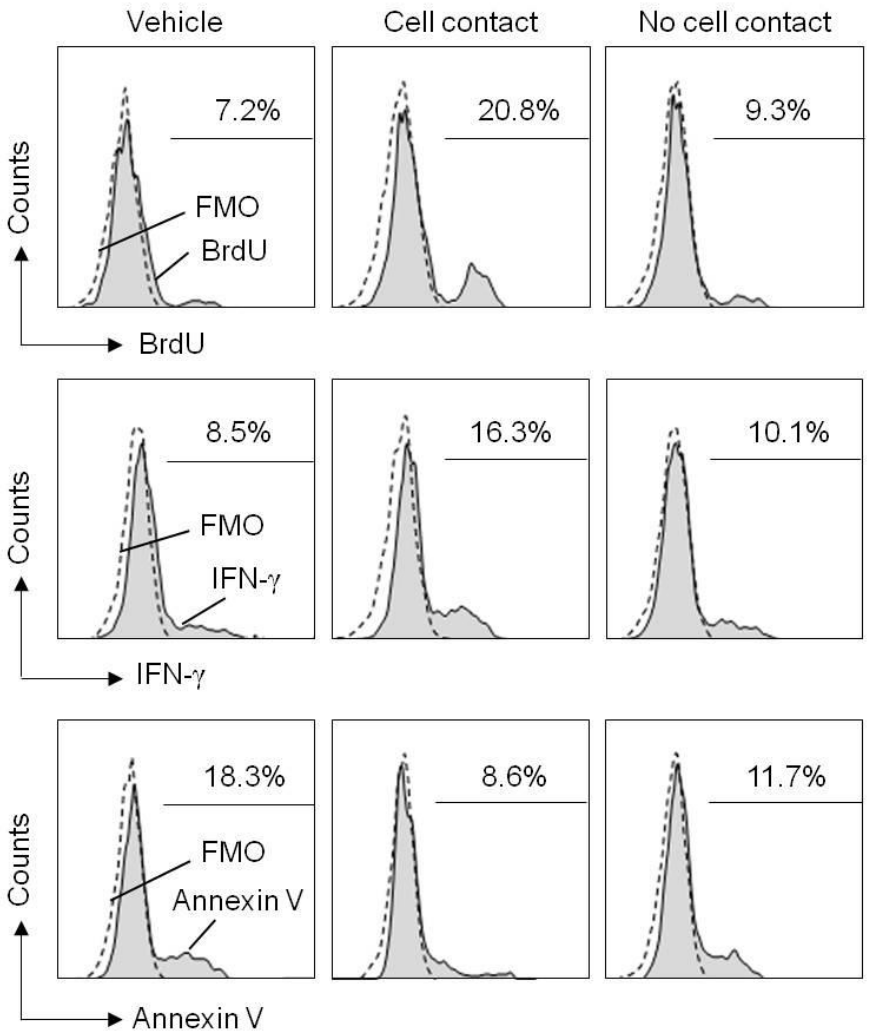
a. ELISA for IL-15, VEGF, IL-4, and MCP-1 from lysates of sorted astrocytes, microglia, neurons and NSCs show that IL-15 is a major factor produced by NSCs as compared to other CNS cells during the recovery phase of EAE (30 dpi). **b-c.** Immunostaining shows that most IL-15-expressing cells in SVZ are $GFAP^+$, whereas only a small portion (<10%) of $IL\text{-}15^+$ cells are microglia/macrophages ($Iba1^+$). Average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 2.3 ± 0.6 at 30 dpi. *n* = 12 per group. Data represent three individual experiments. Error bars represent s.e.m.



Supplementary Figure 5

Removal of IL-15 from NSCs prevents NK cell retention during the late stage of brain inflammation.

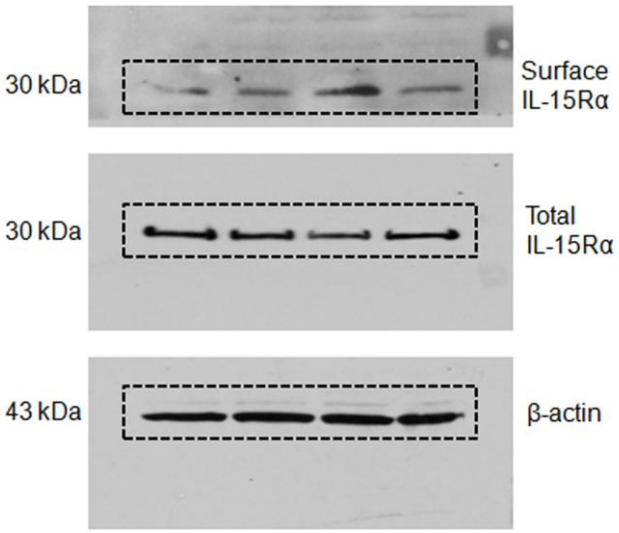
a. Schematic map of the nestin-specific shIL-15 lentiviral vector. **b.** Intraventricular injection of 1 x 10^7 lentivirus particles containing shIL-15 or control sequence into recipient mice. **c.** Intraventricular injection of a lentivirus containing a shIL-15 sequence results in loss of IL-15 in nestin-expressing cells in the SVZ of *Cd1d*$^{-/-}$ mice. $n = 12$ per group. $P < 0.001$, t (22) = 5.58, unpaired t-test. **d.** Intraventricular injection of a lentivirus containing shIL-15 sequence at 20 dpi prevents NK cell retention in SVZ of *Cd1d*$^{-/-}$ mice at EAE 30 dpi, but not in striatum. Absolute numbers of cells per dissected SVZ or striatum tissue are shown. Average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 2.5 ± 0.6 in lenti-control group and 1.7 ± 0.5 in lenti-shIL-15 group at 30 dpi. $n = 12$ per group. $P < 0.001$ (Lenti-control SVZ versus Lenti-control striatum), $P < 0.001$ (Lenti-control SVZ vs. Lenti-sh-IL-15 SVZ). F (1, 44) = 5.5, two-way ANOVA. **e.** To determine BrdU incorporation in NK cells *in vivo*, EAE mice were given i.p. injections of 120 mg kg^{-1} BrdU in PBS daily, and were sacrificed 10 days after the last injection at 30 dpi. Representative flow cytometry plots show that intraventricular injection of a lentivirus containing a shIL-15 sequence reduces BrdU$^+$ NK cell counts in SVZ of *Cd1d*$^{-/-}$ mice at 30 dpi. Bar graphs summarize the decreased counts of BrdU$^+$ NK cells after intraventricular injection of a lentivirus containing a shIL-15 sequence at 30 dpi. Absolute numbers of cells per dissected SVZ tissue are shown. $n = 12$ per group. $P = 0.006$, t (22) = 2.23, unpaired t-test. **f.** Intraventricular injection of a lentivirus containing a shIL-15 sequence at 20 dpi reduces the counts of IFN-γ-expressing NK cells in SVZ of *Cd1d*$^{-/-}$ mice at EAE 30 dpi. Bar graphs summarize the decreased counts of IFN-γ-expressing NK cells after intraventricular injection of a lentivirus containing a shIL-15 sequence at 30 dpi. Absolute numbers of cells per dissected SVZ tissue are shown. $n = 12$ per group. $P = 0.007$, t (22) = 2.11, unpaired t-test. In **e-f**, average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 2.6 ± 0.5 in lenti-control group and 1.8 ± 0.5 in Lenti-shIL-15 group at 30 dpi. Data represent three independent experiments. Error bars represent s.e.m.; ** $P < 0.01$.



Supplementary Figure 6

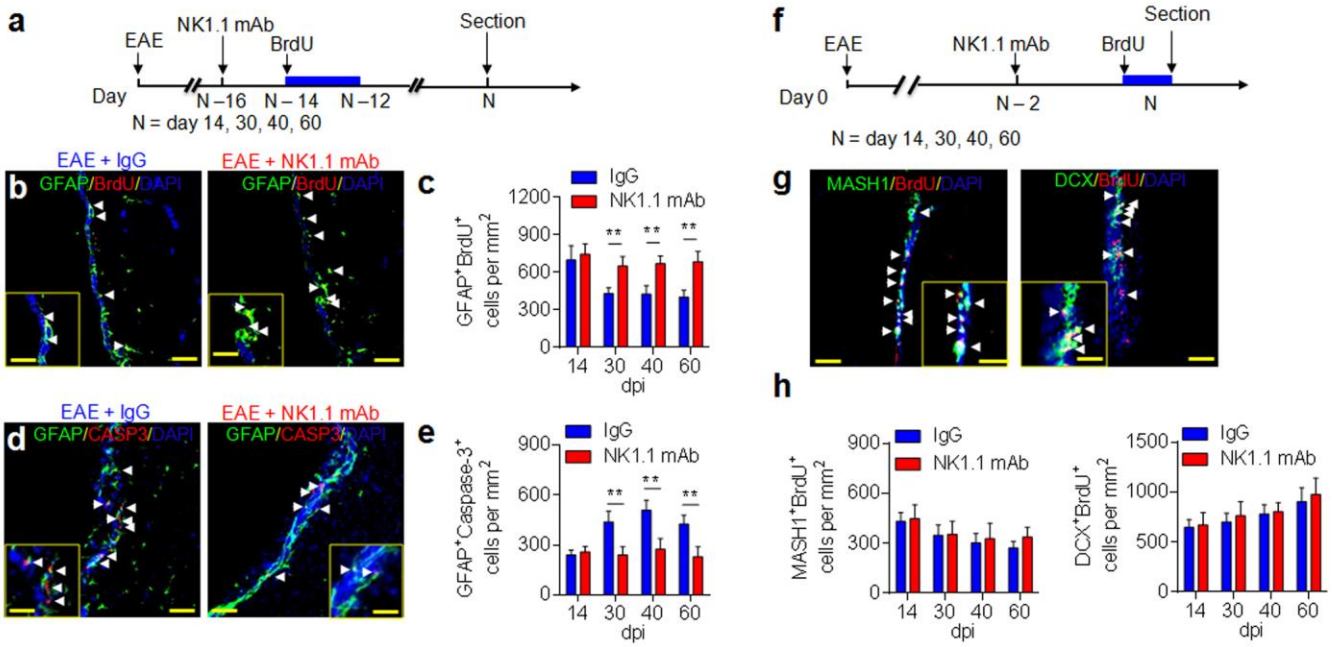
FMO controls and gating strategy for $BrdU^+$, IFN-γ^+ or annexin V^+ NK cells.

NK cells were cultured with flow cytometry-sorted NSCs from EAE SVZ (30 dpi) in transwell plates and counted by flow cytometry in the same well (with cell contact) or spatially separated (no cell contact) by a membrane (0.4 μm pores). Representative flow cytometry dot plots show FMO controls and gating strategy for $BrdU^+$, IFN-γ^+ or annexin V^+ NK cells.



Supplementary Figure 7

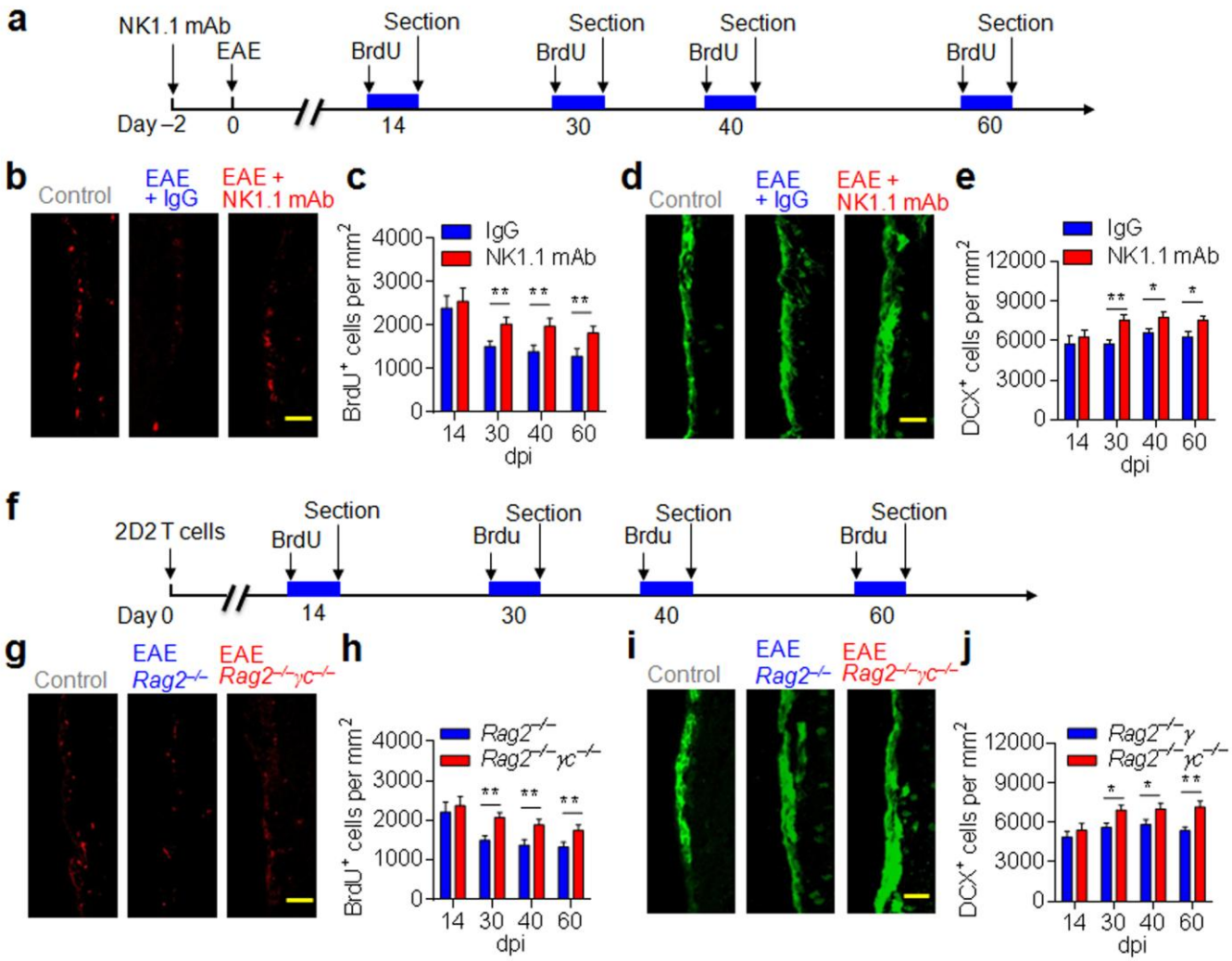
Full-length blots for **Figure 6**.



Supplementary Figure 8

Depletion of NK cells increases the number of proliferating NSCs, but does not affect the numbers of proliferating SVZ type C and A cells during EAE recovery.

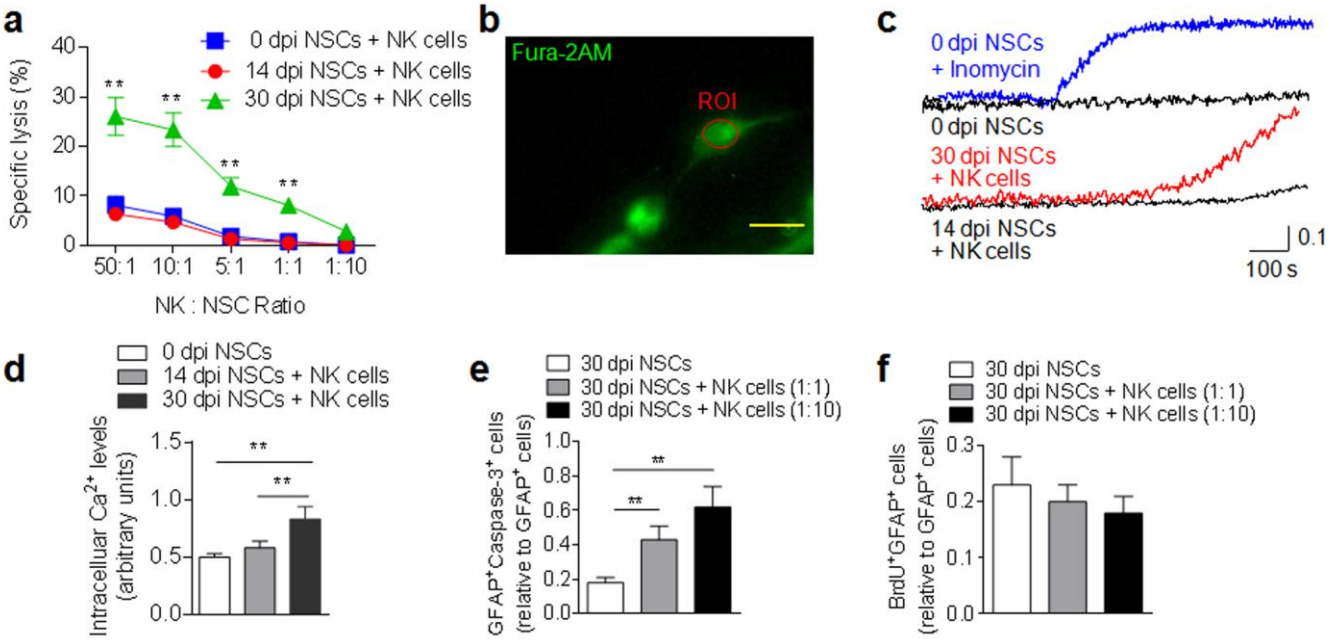
a. "Long-term" BrdU injection was used to determine the number of SVZ-retaining type B cells. Anti-NK1.1 mAb was used to deplete NK cells in *Cd1d*$^{-/-}$ mice devoid of natural killer T (NKT) cells. **b.** Increased numbers of SVZ GFAP^{+}BrdU^{+} cells in EAE *Cd1d*$^{-/-}$ mice treated with anti-NK1.1 mAb as compared to mice injected with IgG control at 30 dpi. **c.** Counts of GFAP^{+}BrdU^{+} cells were increased in EAE *Cd1d*$^{-/-}$ mice treated with NK1.1 mAb during the late stages of EAE (30, 40, 60 dpi) but not in the peak phase (14 dpi). *n* = 6 mice per group at each time point. *P* = 0.003 (30dpi), *P* = 0.002 (40dpi), *P* = 0.005 (60dpi). F (3, 40) = 2.82, two-way ANOVA. **d.** Decreased SVZ GFAP^{+}caspase-3^{+} cells in EAE *Cd1d*$^{-/-}$ mice treated with NK1.1 mAb as compared to those injected with IgG control at 30 dpi. **e.** Counts of GFAP^{+}caspase-3^{+} cells were significantly decreased in EAE *Cd1d*$^{-/-}$ mice treated with NK1.1 mAb as compared to IgG-treated control during the late stages of EAE (30, 40, 60 dpi) but not in the peak phase (14 dpi). *n* = 6 mice per group at each time point. *P* = 0.005 (30dpi), *P* = 0.004 (40dpi), *P* = 0.008 (60dpi). F (3, 40) = 2.23, two-way ANOVA. In **c** and **e**, average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 3.5 ± 0.7 (IgG) and 3.3 ± 0.7 (NK 1.1 mAb) at 14 dpi, 2.6 ± 0.6 (IgG) and 1.6 ± 0.5 (NK 1.1 mAb) at 30 dpi, 2.3 ± 0.6 (IgG) and 1.5 ± 0.5 (NK 1.1 mAb) at 40 dpi and 2.2 ± 0.6 (IgG) and 1.3 ± 0.5 (NK 1.1 mAb) at 60 dpi. **f.** Anti-NK1.1 mAbs were injected after EAE induction, and "short-term" BrdU labeling was used to birthdate rapidly dividing SVZ progenitors in *Cd1d*$^{-/-}$ mice. **g.** Representative images show MASH1^{+}BrdU^{+} or DCX^{+}BrdU^{+} cells in EAE *Cd1d*$^{-/-}$ mice at 30 dpi. **h.** Unchanged counts of MASH1^{+}BrdU^{+} or DCX^{+}BrdU^{+} cells in EAE *Cd1d*$^{-/-}$ mice as compared to IgG-injected controls after pretreatment with anti-NK1.1 mAbs. In **h**, average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 3.2 ± 0.6 (IgG) and 3.3 ± 0.5 (NK 1.1 mAb) at 14 dpi, 2.5 ± 0.7 (IgG) and 1.7 ± 0.5 (NK 1.1 mAb) at 30 dpi, 2.5 ± 0.7 (IgG) and 1.6 ± 0.6 (NK 1.1 mAb) at 40 dpi and 2.3 ± 0.5 (IgG) and 1.5 ± 0.6 (NK 1.1 mAb) at 60 dpi. *n* = 6 mice per group at each time point. Data represent three independent experiments. Scale bars: 40 μm, 20 μm (inset). Error bars represent s.e.m.; ** *P* < 0.01.



Supplementary Figure 9

Removal of NK cells is associated with proliferation of SVZ NSPCs during the late stages of EAE.

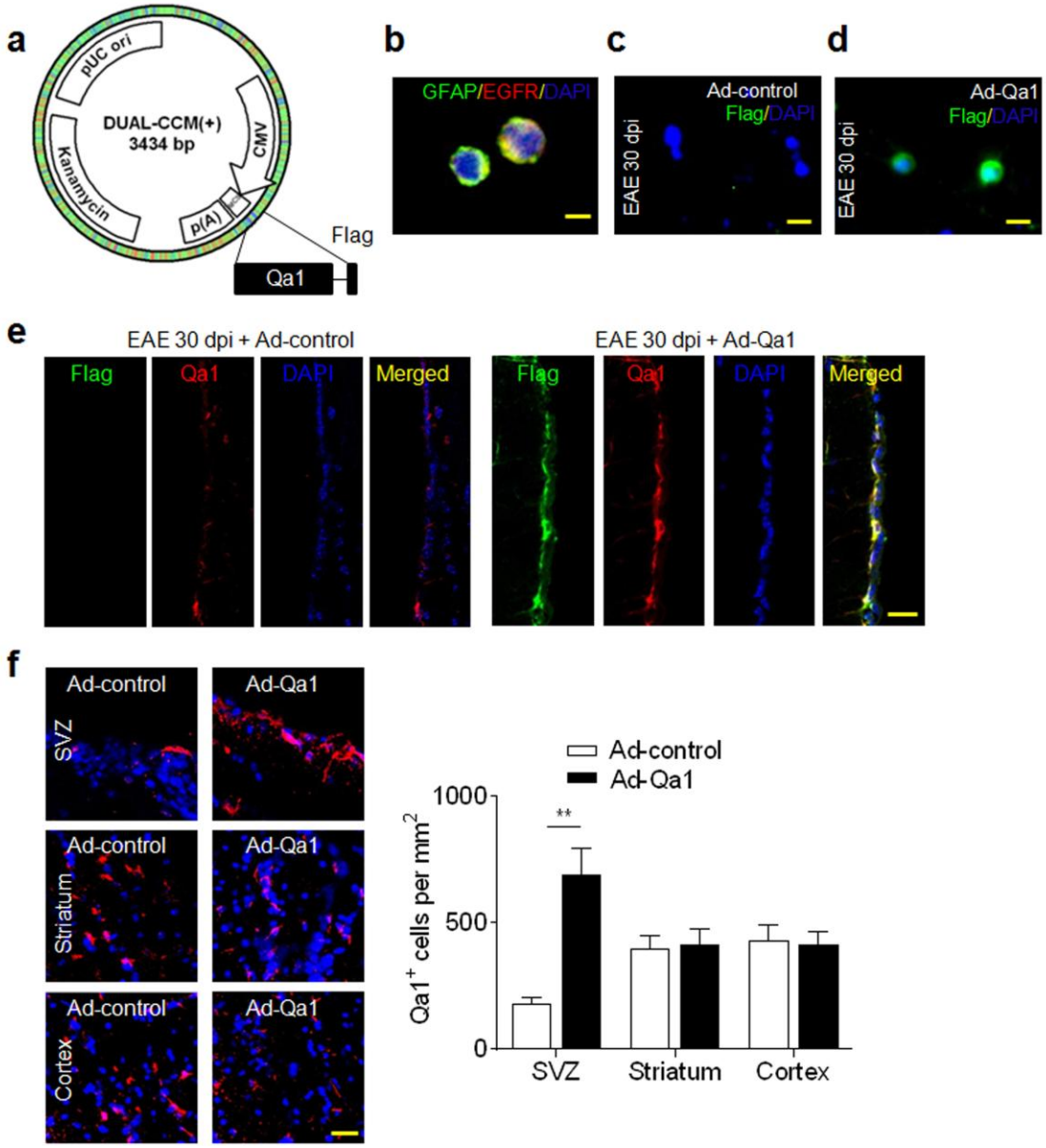
a. Protocol of anti-NK1.1mAb injection, EAE induction and BrdU injection. **b.** Depletion of NK cells using anti-NK1.1 mAb in EAE *Cd1d*$^{-/-}$ mice is associated with increased numbers of BrdU^{+} cells in SVZ at 30 dpi. Control: 0 dpi. **c.** Quantitation of BrdU^{+} cells in EAE *Cd1d*$^{-/-}$ mice pretreated with IgG (control) or anti-NK1.1 mAb. *n* = 6 mice per group at each time point. *P* = 0.008 (30dpi), *P* = 0.006 (40dpi), *P* = 0.007 (60dpi). F (3, 40) = 7.73, two-way ANOVA. **d.** Depletion of NK cells is associated with increased numbers of DCX^{+} cells in SVZ of EAE *Cd1d*$^{-/-}$ mice at 30 dpi. Control: 0 dpi. **e.** Quantitation of DCX^{+} cells in EAE *Cd1d*$^{-/-}$ mice pretreated with IgG or anti-NK1.1 mAb. *n* = 6 mice per group at each time point. *P* = 0.007 (30dpi), *P* = 0.012 (40dpi), *P* = 0.013 (60dpi). F (3, 40) = 5.35, two-way ANOVA. In **b-e**, average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 3.5 ± 0.7 (IgG) and 3.3 ± 0.7 (NK 1.1 mAb) at 14 dpi, 2.7 ± 0.5 (IgG) and 1.8 ± 0.7 (NK 1.1 mAb) at 30 dpi, 2.6 ± 0.8 (IgG) and 1.5 ± 0.8 (NK 1.1 mAb) at 40 dpi and 2.5 ± 0.6 (IgG) and 1.3 ± 0.5 (NK 1.1 mAb) at 60 dpi. **f.** EAE induction and BrdU injection in *Rag2*$^{-/-}$or *Rag2*$^{-/-}\gamma c^{-/-}$ *Cd1d*$^{-/-}$ mice that received 2D2 *Cd1d*$^{-/-}$ T cells (devoid of NKT cells). **g.** Deletion of NK cells prevents reduction of SVZ-proliferating (BrdU^{+}) cells independently of T, B, and NKT cells at 30 dpi. Control: 0 dpi. **h**. Quantitation of BrdU^{+} cells in SVZ from *Rag2*$^{-/-}$ and *Rag2*$^{-/-}\gamma c^{-/-}$ mice during EAE. *n* = 6 mice per group at each time point. *P* = 0.008 (30dpi), *P* = 0.006 (40dpi), *P* = 0.008 (60dpi). F (3, 40) = 8.36, two-way ANOVA. **i**. Deficiency of NK cells is associated with increased numbers of DCX^{+} cells in *Rag2*$^{-/-}$ and *Rag2*$^{-/-}\gamma c^{-/-}$ mice at 30 dpi. Control: 0 dpi. **j.** Quantitation of DCX^{+} cells in SVZ from *Rag2*$^{-/-}$ and *Rag2*$^{-/-}\gamma c^{-/-}$ mice. *n* = 6 mice per group at each time point. *P* = 0.043 (30dpi), *P* = 0.036 (40dpi), *P* = 0.009 (60dpi). F (3, 40) = 5.65, two-way ANOVA. In **g-j**, average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 3.2 ± 0.6 (*Rag2*$^{-/-}$) and 3.4 ± 0.7 (*Rag2*$^{-/-}\gamma c^{-/-}$) at 14 dpi, 2.5 ± 0.8 (*Rag2*$^{-/-}$) and 1.7 ± 0.7 (*Rag2*$^{-/-}\gamma c^{-/-}$) at 30 dpi, 2.3 ± 0.7 (*Rag2*$^{-/-}$) and 1.5 ± 0.8 (*Rag2*$^{-/-}\gamma c^{-/-}$) at 40 dpi and 2.2 ± 0.6 (*Rag2*$^{-/-}$) and 1.3 ± 0.5 (*Rag2*$^{-/-}\gamma c^{-/-}$) at 60 dpi. Experiments were repeated three times. Scale bars: 40 μm. Error bars represent s.e.m.; * *P* < 0.05; ** *P* < 0.01.



Supplementary Figure 10

NK cell–induced cytotoxicity against SVZ type B cells.

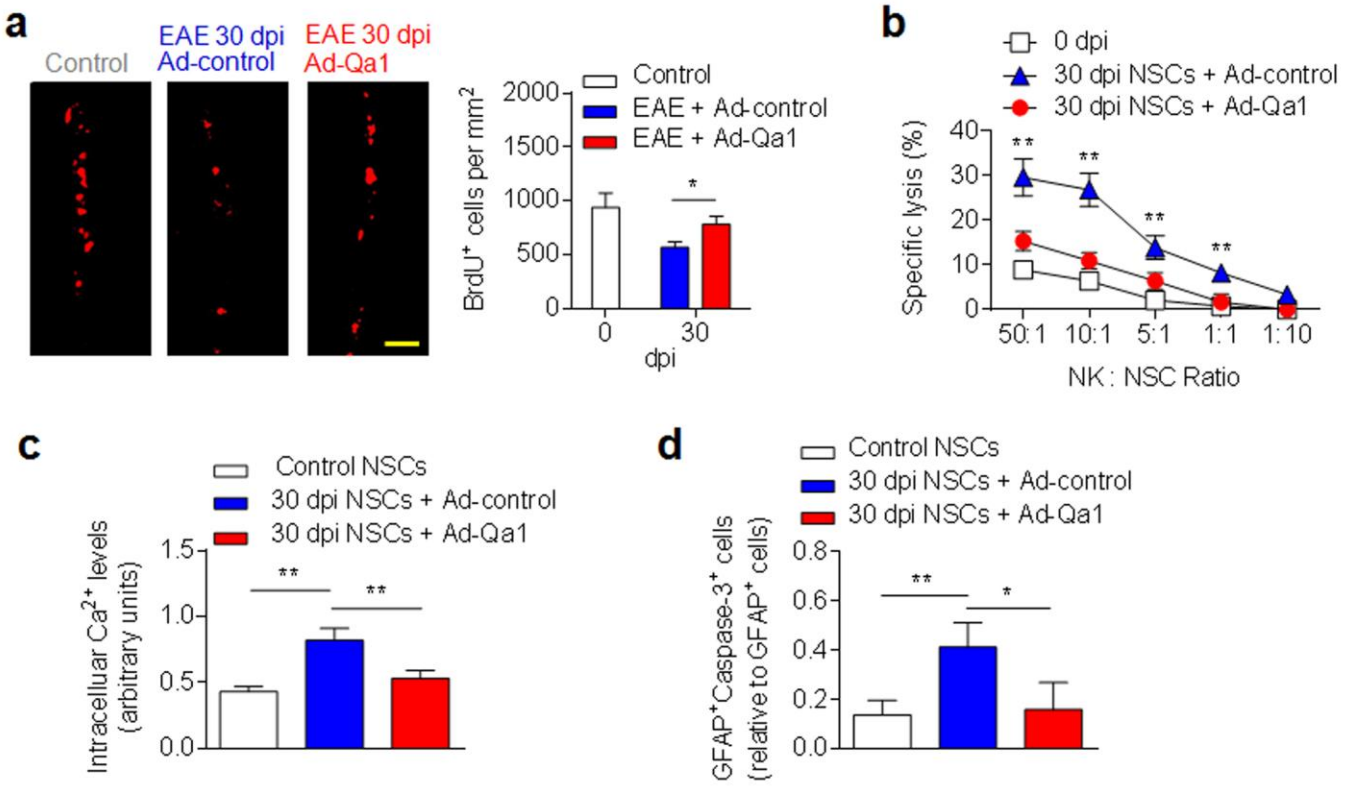
a. Cytotoxicity against NSCs by NK cells was measured by ^{51}Cr-release assay. Target cells (cultured NSCs) were labeled with ^{51}Cr. 10^4 NSCs/well from naïve control, EAE 14 dpi and EAE 30 dpi SVZ, were co-cultured with activated NK cells at different effector:target ratios (50:1, 10:1; 5:1; 1:1, and 1:10) for 24 h. Activated NK cells were prepared by IL-2 (10 µg ml^{-1}), IL-15 (10 µg ml^{-1}), and LPS (5 µg ml^{-1}) stimulation for 96 h prior to co-culture. Cytotoxicity was measured and data shown are expressed as percentage of naïve controls from four independent experiments with each value repeated in triplicate. Average EAE disease grade for mice used for NSC isolation at the time of tissue harvesting was 3.6 ± 0.7 at 14 dpi and 2.6 ± 0.6 at 30 dpi. n = 12 per group. $P < 0.001$ (50:1), $P < 0.001$ (10:1), $P < 0.001$ (5:1), $P < 0.001$ (1:1). F (8, 165) = 6.26, two-way ANOVA. **b.** A representative image of Fura-2AM-loaded NSCs and selected region of interest (ROI). Scale bar, 20 µm. **c.** Increased Ca^{2+} influx signals in NSCs isolated from EAE SVZ at 30 dpi and treated with activated NK cells. Ca^{2+} influx signals were measured by the ratio of 340/380nm in the selected ROIs of NSCs. **d.** Averaged Ca^{2+} influx signals in ROIs selected from NSCs from the indicated groups, which were treated or untreated with activated NK cells for 12 h. Average EAE disease grade for mice used for NSC isolation at the time of tissue harvesting was 3.5 ± 0.6 at 14 dpi and 2.3 ± 0.8 at 30 dpi. n = 8 per group. $P = 0.005$ (30 dpi NSCs + NK cells versus 0 dpi NSCs), $P = 0.008$ (30 dpi NSCs + NK cells versus 14 dpi NSCs + NK cells). F (2, 21) = 2.78, one-way ANOVA. In **a-d**, data represent four independent experiments. **e.** NSCs were sorted from EAE SVZ at 30 dpi by flow cytometry and cultured *in vitro* for 48 h. Immunostaining shows increased counts of GFAP$^+$caspase-3$^+$ cells relative to total GFAP$^+$ cells after treatment with NK cells for 24 h. n = 12 per group. $P = 0.006$ (30 dpi NSCs + NK cells 1:1 versus 30 dpi NSCs), $P = 0.003$ (30 dpi NSCs + NK cells 1:10 versus 30 dpi NSCs). F (2, 33) = 5.88, one-way ANOVA. **f.** Cell proliferation was determined by *in vitro* BrdU incorporation and co-labeling with GFAP. Counts of BrdU$^+$GFAP$^+$ cells relative to total GFAP$^+$ cells in NSC cultures were unaffected by treatment with NK cells. Results in **e-f** are from three independent experiments. n = 12 per group. Average EAE disease grade for mice used for NSC isolation at the time of tissue harvesting was 2.5 ± 0.6 at 30 dpi. Error bars represent s.e.m.; ** $P < 0.01$.



Supplementary Figure 11

Construction of and treatment with adenoviral vectors encoding Qa1.

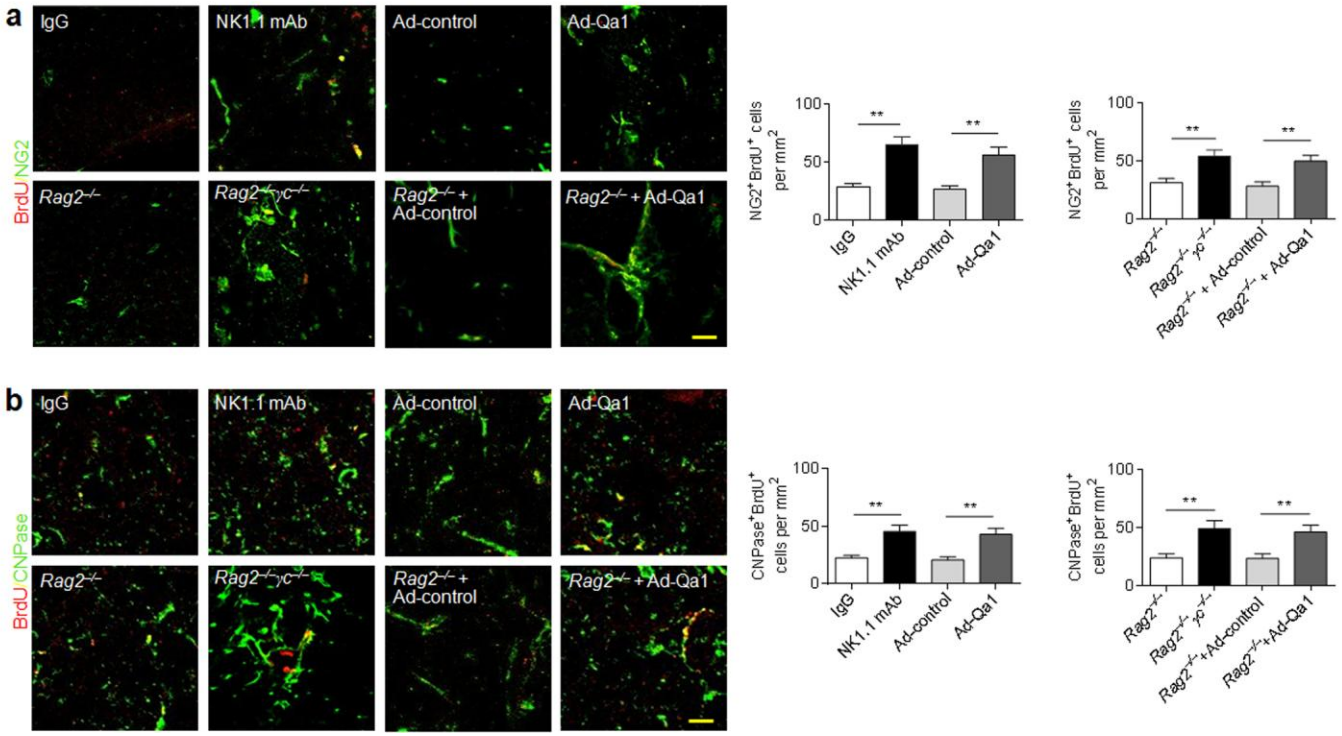
a. Full-length Qa1 cDNA was amplified by RT-PCR from RNA isolated from splenocytes of C57BL/6 mice. After verification of the sequence, cDNA was ligated into the DUAL-CCM^{+} shuttle vector with a C-terminal Flag epitope tag under the control of a CMV promoter. The recombinant adenovirus was custom generated by Vector Biolabs. **b.** A representative image of SVZ type B cells (GFAP^{+}EGFR^{+}DAPI^{+}) freshly isolated and sorted by flow cytometry from *Cd1d*$^{-/-}$ mice at 30 dpi. Scale: 10 μm. **c−d.** SVZ type B cells freshly isolated and sorted by flow cytometry from *Cd1d*$^{-/-}$ mice at 30 dpi were cultured prior to infection with 1X10^{7} pfu adenovirus without Qa1 sequence (control, **c**) or 1X10^{7} pfu adenovirus containing Qa1 sequence (Ad-Qa1, **d**). Cells were fixed, permeabilized, blocked with anti-Flag Ab and stained with an Alexa Fluor secondary Ab. Scale: 20 μm. **e.** Intraventricular injection of adenovirus containing Qa1 sequence at 20 dpi prevents loss of Qa1 expression in SVZ of *Cd1d*$^{-/-}$ mice at 30 dpi, Scale: 50 μm. **f.** Intraventricular injection of adenovirus containing Qa1 sequence at 20 dpi prevents loss of Qa1 expression in SVZ, but not in striatum (STM) or cortex (CTX) of *Cd1d*$^{-/-}$ mice at 30 dpi, Scale: 40 μm. n = 12 mice per group. P = 0.001 (Ad-control SVZ versus Ad-Qa1 SVZ), t (22) = 3.83, unpaired t-test. In **e-f**, average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 2.6 ± 0.5 in Ad-control group and 1.5 ± 0.5 in Ad-Qa1 group at 30 dpi. Data represent three independent experiments. Error bars represent s.e.m.; ** P < 0.01.



Supplementary Figure 12

Qa1 overexpression in the SVZ promotes the survival of NSCs during the late stages of brain inflammation.

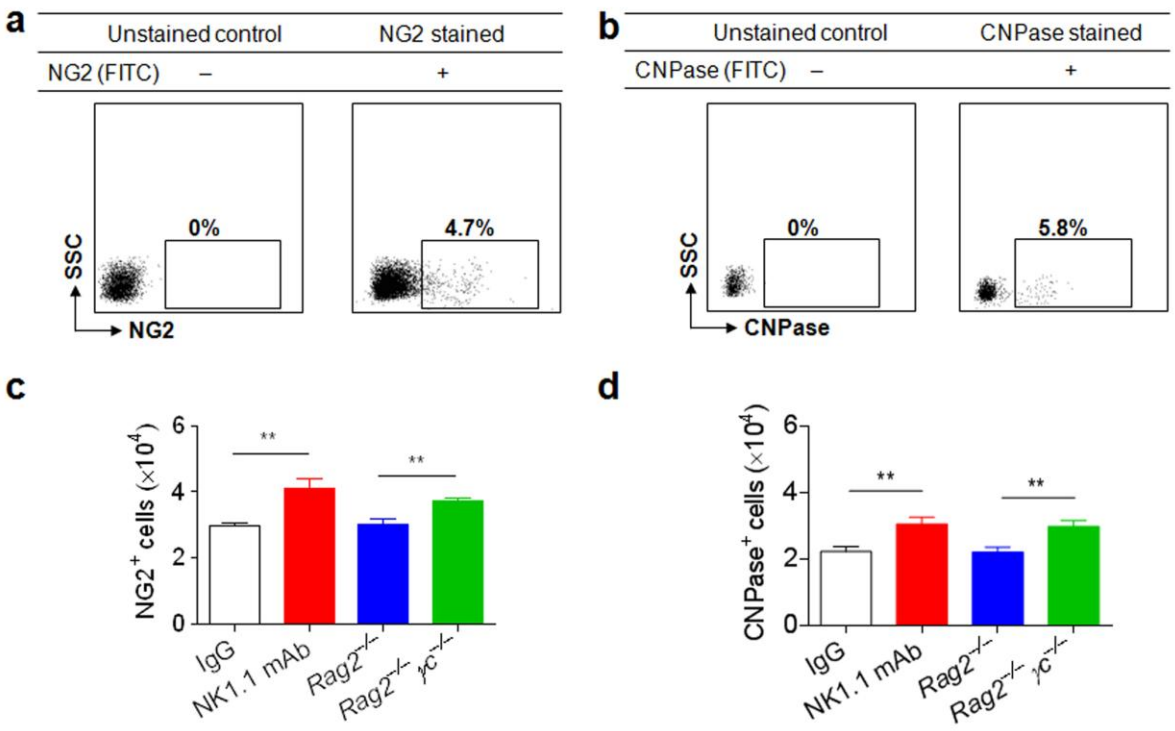
a. "Long-term" BrdU injection was used to determine the number of SVZ-retaining type B cells. Representative images and bar graph show increased $BrdU^+$ cells in EAE SVZ (30 dpi) of *Cd1d*$^{-/-}$ mice receiving intraventricular injection of adenovirus containing Qa1 sequence at 20 dpi. EAE mice receiving adenovirus containing null control sequence were used as control (Ad-control). Control: 0 dpi. Average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 2.4 ± 0.5 in Ad-control group and 1.5 ± 0.4 in Ad-Qa1 group at 30 dpi. Scale: 50 μm. n = 12 mice per group. P = 0.02 (EAE + Ad-control versus EAE + Ad-Qa1), t (22) = 2.42, unpaired t-test. **b.** Cytotoxicity against NSCs by NK cells was measured by ^{51}Cr-release assay. Target cells (NSCs) were obtained from naïve control SVZ, EAE 30 dpi SVZ from *Cd1d*$^{-/-}$ mice receiving intraventricular injection of adenovirus containing Qa1 or null control sequence at 20 dpi. Target cells were labeled with ^{51}Cr. 10^4 NSCs/well were then co-cultured with activated NK cells at different effector:target ratios (50:1, 10:1; 5:1; 1:1, and 1:10) for 24 h. Activated NK cells were prepared by IL-2 (10 μg ml^{-1}), IL-15 (10 μg ml^{-1}), and LPS (5 μg ml^{-1}) stimulation for 96 h prior to co-culture. Cytotoxicity was measured and data shown are expressed as percentage of naïve controls from four independent experiments with each value repeated in triplicate. Average EAE disease grade for mice used for NSC isolation at the time of tissue harvesting was 2.6 ± 0.7 in Ad-control group and 1.4 ± 0.6 in Ad-Qa1 group at 30 dpi. n = 12 per group. P < 0.001 (50:1), P < 0.001 (10:1), P < 0.001 (5:1), P < 0.001 (1:1). F (8, 165) = 3.73, two-way ANOVA. **c.** Qa1 overexpression attenuates the increase of Ca^{2+} influx signals in NSCs treated with activated NK cells. Ca^{2+} influx signals were measured by the ratio of 340/380nm in the selected ROIs of NSCs. NSCs were obtained from naïve control SVZ, EAE 30 dpi SVZ of *Cd1d*$^{-/-}$ mice receiving intraventricular injection of adenovirus containing Qa1 or null control sequence at 20 dpi. Averaged Ca^{2+} influx signals in ROIs selected from NSCs from the indicated groups, which were treated with activated NK cells for 12 h. Average EAE disease grade for mice used for NSC isolation at the time of tissue harvesting was 2.5 ± 0.6 in Ad-control group and 1.5 ± 0.5 in Ad-Qa1 group at 30 dpi. Control: 0 dpi. n = 8 per group. P = 0.003 (30 dpi NSCs + Ad-control versus Control), P = 0.006 (30 dpi NSCs +Ad-control versus 30 dpi NSCs + Ad-Qa1). F (2, 21) = 9.37, one-way ANOVA. **d.** NSCs were sorted from naïve control SVZ, EAE 30 dpi SVZ of *Cd1d*$^{-/-}$ mice receiving intraventricular injection of adenovirus containing Qa1 or null control sequence at 20 dpi. Sorted NSCs were then cultured *in vitro* for 48 h. Immunostaining shows that Qa1 overexpression reduced the increase of $GFAP^+$caspase-3^+ cell counts relative to total $GFAP^+$ cells after treatment with NK cells for 24 h. Control: 0 dpi. Average EAE disease grade for mice used for NSC isolation at the time of tissue harvesting was 2.3 ± 0.5 in Ad-control group and 1.4 ± 0.4 in Ad-Qa1 group at 30 dpi. n = 12 per group. P = 0.003 (30 dpi NSCs + Ad-control versus Control), P = 0.02 (30 dpi NSCs + Ad-control versus 30 dpi NSCs + Ad-Qa1). F (2, 33) = 3.12, one-way ANOVA. Data represent four independent experiments. Error bars represent s.e.m.; * P < 0.05, ** P < 0.01.



Supplementary Figure 13

Removal of NK cells or overexpression of Qa1 in the SVZ promotes oligodendrogenesis around the SVZ.

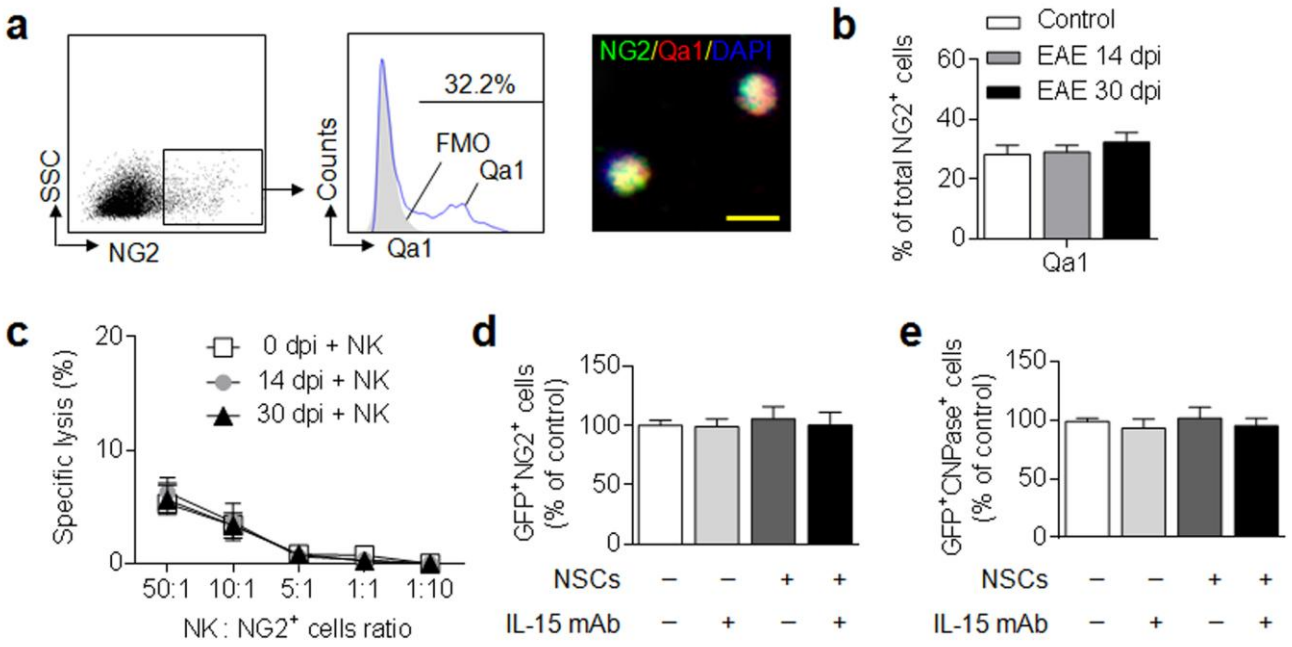
EAE was induced in *Cd1d*$^{-/-}$, *Rag2*$^{-/-}$ and *Rag2*$^{-/-}\gamma c^{-/-}$ mice. To induce EAE, *Rag2*$^{-/-}$ and *Rag2*$^{-/-}\gamma c^{-/-}$ mice were transferred with 5X10^6 2D2 T cells on day 0. To deplete NK cells, *Cd1d*$^{-/-}$ mice received anti-NK1.1 mAb injection (i.p.) at two days prior to MOG immunization and every 5 days thereafter until termination of experiments. EAE mice received intraventricular injection of adenovirus containing Qa1 or null control sequence at 20 dpi and SVZ tissues from EAE mice were harvested at 30 dpi. **a.** To determine the generation of neural/glial antigen 2 (NG2)/ BrdU$^+$ oligodendrocyte progenitors, EAE mice were given i.p. injections of 120 mg kg^{-1} BrdU in PBS every 2 h for 10 h, and were sacrificed 1 week (7 d washout time) after the last injection at 30 dpi. Quantitation of BrdU$^+$NG2$^+$ cells in the SVZ of EAE mice (30 dpi) is expressed as the total number of positive cells per area measured (cells per mm^2). *n* = 12 mice per group. *P* = 0.004 (IgG versus NK1.1 mAb), t (22) = 2.36. *P* = 0.004 (Ad-control versus Ad Qa1), t (22) = 2.28. *P* = 0.006 (*Rag2*$^{-/-}$ versus *Rag2*$^{-/-}\gamma c^{-/-}$), t (22) = 2.03. *P* = 0.007 (*Rag2*$^{-/-}$ + Ad-control versus *Rag2*$^{-/-}$+ Ad-Qa1), t (22) = 1.96, unpaired t-test. **b.** To determine newly generated mature cyclic nucleotide phosphohydrolase (CNPase)/ BrdU$^+$ oligodendrocytes, mice were injected i.p. with 150 mg kg^{-1} BrdU twice per day for 2 days and were killed 2 weeks (14 d washout time) after the last injection. Quantitation of BrdU$^+$CNPase$^+$ cells in the SVZ of EAE mice (30 dpi) is expressed as the total number of positive cells per area measured (cells per mm^2). In **a-b**, average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 2.6 ± 0.6 in IgG group, 1.3 ± 0.5 in NK1.1-mAb group, 2.5 ± 0.5 in Ad-control group, 1.4 ± 0.6 in Ad-Qa1 group, 2.3 ± 0.5 in *Rag2*$^{-/-}$ group, 1.2 ± 0.6 in *Rag2*$^{-/-}\gamma c^{-/-}$ group, 2.4 ± 0.7 in *Rag2*$^{-/-}$ + Ad-control group, 1.3 ± 0.6 in *Rag2*$^{-/-}$ + Ad-Qa1 group at 30 dpi, Scale bars: 40 μm. *n* = 12 mice per group. *P* = 0.003 (IgG versus NK1.1 mAb), t (22) = 2.83. *P* = 0.003 (Ad-control versus Ad Qa1), t (22) = 3.01. *P* = 0.007 (*Rag2*$^{-/-}$ versus *Rag2*$^{-/-}\gamma c^{-/-}$), t (22) = 2.05. *P* = 0.006 (*Rag2*$^{-/-}$ + Ad-control versus *Rag2*$^{-/-}$+ Ad-Qa1), t (22) = 2.12, unpaired t-test. Data represent three independent experiments. Error bars represent s.e.m.; ** *P* < 0.01.



Supplementary Figure 14

Removal of NK cells promotes oligodendrogenesis in the spinal cord.

EAE was induced in *Cd1d*$^{-/-}$, *Rag2*$^{-/-}$ and *Rag2*$^{-/-}\gamma c^{-/-}$ mice. To deplete NK cells, *Cd1d*$^{-/-}$ mice received anti-NK1.1 mAb injection (i.p.) at two days prior to MOG immunization and every 5 days thereafter until termination of experiments. Spinal cord tissues were obtained at 30 dpi from EAE mice. Counts of NG2$^+$ or CNPase$^+$ cells were measured by flow cytometry analysis. **a.** Representative flow cytometry plots show the identification of gating boundaries of NG2 (FITC) with corresponding FMO control. **b.** Representative flow cytometry plots show the identification of gating boundaries of CNPase (FITC) with corresponding FMO control. **c.** Quantitation of NG2$^+$ cells in the spinal cord of EAE mice (30 dpi) is expressed as the total number of positive cells per mouse spinal cord. n = 12 mice per group. P = 0.006 (IgG versus NK1.1 mAb), t (22) = 2.06. P = 0.008 (*Rag2*$^{-/-}$ versus *Rag2*$^{-/-}\gamma c^{-/-}$), t (22) = 1.92, unpaired t-test. **d.** Quantitation of CNPase$^+$ cells in the spinal cord of EAE mice (30 dpi) is expressed as the total number of positive cells per mouse spinal cord. n = 12 mice per group. P = 0.007 (IgG versus NK1.1 mAb), t (22) = 1.98. P = 0.006 (*Rag2*$^{-/-}$ versus *Rag2*$^{-/-}\gamma c^{-/-}$), t (22) = 2.23, unpaired t-test. In **c-d**, average EAE disease grade for mice used for tissue analysis at the time of tissue harvesting was 2.7 ± 0.6 in IgG group, 1.5 ± 0.6 in NK1.1-mAb group, 2.4 ± 0.6 in *Rag2*$^{-/-}$ group and 1.3 ± 0.4 in *Rag2*$^{-/-}\gamma c^{-/-}$ group at 30 dpi. Data represent three independent experiments. Error bars represent s.e.m.; ** P < 0.01.



Supplementary Figure 15

Oligodendrocyte progenitors are resistant to NK cell killing.

a. Representative flow cytometry plots and immunostained image of oligodendrocyte progenitor cells show that oligodendrocyte progenitor cells express Qa1. Scale bar, 20 μm. **b.** Quantitation of $Qa1^+$ oligodendrocyte progenitor cells shows unaltered expression of Qa1 in WT controls (0 dpi) or EAE mice at 14 or 30 dpi. Average EAE disease grade for mice used for cell isolation at the time of tissue harvesting was 3.3 ± 0.8 at 14 dpi and 2.6 ± 0.7 at 30 dpi. *n* = 6 mice per data plot. Results were obtained from three independent experiments. **c.** Cytotoxicity against oligodendrocyte progenitor cells by NK cells was measured by ^{51}Cr-release assay. Target cells (oligodendrocyte progenitor cells) were obtained from naïve control, EAE 14 or 30 dpi CNS tissues from WT mice by sorting $NG2^+$ cells from CNS tissues. Target cells were labeled with ^{51}Cr. 1 x 10^4 oligodendrocyte progenitor cells s/well were then co-cultured with activated NK cells at different effector:target ratios (50:1, 10:1; 5:1; 1:1, and 1:10) for 24 h. Activated NK cells were prepared by IL-2 (10 μg ml^{-1}), IL-15 (10 μg ml^{-1}), and LPS (5 μg ml^{-1}) stimulation for 96 h prior to coculture. Cytotoxicity was measured and data shown are expressed as percentage of naïve controls from four independent experiments with each value repeated in triplicate. Average EAE disease grade for mice used for cell isolation at the time of tissue harvesting was 3.5 ± 0.7 at 14 dpi and 2.5 ± 0.8 at 30 dpi. *n* = 12 per group. **d.** NSCs were sorted from SVZ of WT mice and oligodendrocyte progenitor cells ($NG2^+$) were sorted from UBC-GFP mice respectively by flow cytometry at 30 dpi. Thereafter, oligodendrocyte progenitor cells and NSCs were co-cultured with or without IL-15 mAb *in vitro* for 48 h. Oligodendrocyte progenitor cells cultured alone without IL-15 mAb treatment were used as controls. Immunostaining shows unaltered counts of GFP^+NG2^+ cells relative to those in controls after treatment with IL-15 mAb or co-cultured with NSCs. *n* = 12 per group. **e.** NSCs were sorted from SVZ of WT mice and oligodendrocytes ($CNPase^+$) were sorted from UBC-GFP mice respectively by flow cytometry at 30 dpi. Thereafter, oligodendrocytes and NSCs were co-cultured with or without IL-15 mAb *in vitro* for 48 h. Oligodendrocytes cultured alone without IL-15 mAb treatment were used as controls. Immunostaining shows unaltered counts of $GFP^+CNPase^+$ cells relative to those in controls after treatment with IL-15 mAb or co-cultured with NSCs. *n* = 12 per group. Average EAE disease grade for mice used for cell isolation at the time of tissue harvesting was 3.5 ± 0.8 (WT) and 3.2 ± 0.7 (UBC-GFP) at 14 dpi, 2.5 ± 0.7 (WT) and 2.3 ± 0.8 (UBC-GFP) at 30 dpi. Results were obtained from four independent experiments. Error bars represent s.e.m.