Supporting Information

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SI Methods

Animals. A total of 279 mice, either males or females between 8 and 12 wk of age, were used in this study. C57BL/6 and CD45.1 mice were purchased from Charles River. C57BL/6J and IL-1R1–KO were purchased from The Jackson Laboratory. All of the genetically modified mouse strains described below were bred in-house at the Animal Research Facility of the Centre de recherche du Centre hospitalier universitaire de Québec–Université Laval (Pavillon du CHUL). Mice transgenic for a TCR specific for the $MOG_{35–55}$ peptide (referred hereafter as 2D2 mice) and β-actin–GFP transgenic mice were initially purchased from The Jackson Laboratory. Breeders for IL-1β–KO and LysM-eGFP knock-in mice were respectively obtained from Y. Iwakura, Institute of Medical Science, University of Tokyo, Japan, and G. Dekaban, Robarts Research Institute, London, Ontario, Canada, with prior authorization from T. Graf, Barcelona, Spain. pIl1b-DsRed transgenic mice were obtained from A. Takashima, University of Toledo, Toledo, OH. All mice had ad libidum access to food and water.

Production of Mixed Bone Marrow Chimeras. To destroy hematopoietic stem cells, female recipient mice (CD45.1) aged 8–10 wk were exposed to a total body γ-irradiation with a single dose of 7.5 Gy using a cesium-173 source (Gammacell 40 Exactor, MDS Nordion). Recipients were then injected with a 50/50 mix of β-actin–GFP and *Il1b*$^{-/-}$ freshly isolated bone marrow cells (a total of 10×10^6) via the tail vein, as described before (5). Briefly, femurs and tibias were harvested from euthanized donor mice and flushed with HBSS (without Ca^{2+}/Mg^{2+}) + 2% FBS using a 25-gauge needle. After the bone marrow transplantation, mice were kept in sterile cages and treated for 2 wk with antibiotics [2.5 mL of Septra (GlaxoSmithKline) in 200 mL of drinking water]. Chimerism was measured by flow cytometry at 8 wk posttransplant (92%), after which animals were immunized.

EAE Induction and Clinical Scoring. Mice were immunized by s.c. injection of 100 μg of $MOG_{35–55}$ (MEV GWY RSP FSR VVH LYR NGK, Feldan) emulsified in incomplete Freund's adjuvant (BD Biosciences) supplemented with 4 mg/mL heat-inactivated *Mycobacterium tuberculosis* H37Ra (BD Biosciences). Pertussis toxin (200 ng per mouse; List Biological Laboratories Inc.) was injected in the tail vein at immunization and again 2 d later. Animals were monitored daily for weight loss and scored using a 5-point grading method according to the recommendation of Stromnes and Goverman (38), as previously reported (5). Mice were killed by exsanguination or by anesthetic overdose at different days postimmunization, as indicated.

Biological Sample Collection and Processing for Cytometry.

Blood. Animals were anesthetized with a mixture of 400 mg/kg ketamine and 40 mg/kg xylazine. Blood was collected via cardiac puncture using a 22-gauge syringe and immediately transferred into EDTA-coated microtubes (Sarstedt). Blood samples were then put on slow rotation at room temperature until processing.

Bone marrow. Animals were anesthetized as described above and their left femurs isolated and flushed with HBSS (without Ca^{2+}/Mg^{2+}) + 2% FBS using a 25-gauge needle. Erythrocytes were lysed using the ACK buffer (150 mM NH_4Cl, 10 mM potassium bicarbonate, 0.01 mM EDTA). Cells were manually counted with a hemocytometer (Hausser Scientific).

Spleen. Spleens were harvested from anesthetized animals and placed in HBSS (without Ca^{2+}/Mg^{2+}). Spleens were homogenized and passed through a 70-μm nylon mesh strainer (BD Biosciences). Erythrocyte lysis was performed using the ACK buffer. The cell suspension was passed on a second 70-μm nylon mesh strainer and the cell count measured.

Spinal cord. Anesthetized mice were perfused with ice-cold HBSS for 2 min and their spinal cord flushed with an 18G BD PrecisionGlide needle. Spinal cords were homogenized using a PTFE tissue grinder and enzymatically digested for 30 min at 37 °C, as previously described (5). Briefly, the enzymatic mixture contained 0.25% collagenase IV (Worthington Biochemical Corporation), 1 U/mL elastase (Worthington Biochemical Corporation), 0.025 U/mL DNase I (Worthington Biochemical Corporation), 0.1 μg/mL Nα-Tosyl-l-lysine chloromethyl ketone hydrochloride (Sigma-Aldrich), and 20 mM Hepes in HBSS in a final volume of 3 mL per tissue. Spinal cords were passed through a 70-μm nylon mesh strainer, placed on a 37%/70% Percoll gradient (GE Healthcare), and centrifuged for 20 min at 1,000 × *g* (without acceleration or break). Immune cells at the interface were collected, washed in PBS, and used for staining.

Neutrophil Transfer. Neutrophils were isolated from the bone marrow of immunized C57BL/6 mice as reported before (5). Briefly, bone marrow was flushed with HBSS + 2% FBS with a 25-gauge needle. Neutrophils were isolated using two consecutive Percoll gradients of 64.8% and 61.5%, washed, and then resuspended in sterile PBS at a concentration of 5×10^7 cells per milliliter. On days 3, 5, and 7 postimmunization, 5×10^6 neutrophils were injected i.p. to WT and *Il1b*$^{-/-}$ mice.

Flow Cytometry. A volume of 100 μL (blood) or a total of 1.5×10^6 cells (spleen and bone marrow) were used for each staining. For the flow cytometry analysis of the spinal cords, the entire tissue was used. Fragment crystallizable receptors were blocked with an anti-CD16/CD32 mix (BD Biosciences) in KPBS + 0.5% FBS for 15 min. Leukocytes were stained for 30 min with primary antibodies (see Table S1 for the complete list). For blood samples, erythrocytes were lysed after the antibody incubation using Red Blood Cell Lysis Buffer (Beckman Coulter). For cytokine intracellular staining, mononuclear cells isolated from the spinal cord as described above were cultured for 4 h in GolgiStop (1 μL/mL culture; BD Biosciences) and stimulated with 50 ng/mL of phorbol 12-myristate 13-acetate (Sigma-Aldrich) and 1 μM of ionomycin (Sigma-Aldrich), as before (39). Cells were then incubated with Fc Block and stained for cell surface markers and Live/Dead eFluor506 (eBioscience). They were then fixed and permeabilized for 20 min with the Fixation and Permeabilization Wash Buffer (BioLegend) before adding antibodies directed against IFNγ, IL-17A, and GM-CSF.

For all analyses, debris, remaining erythrocytes, and doublets were discarded according to their forward and side scatter characteristics. Fluorescence-minus-one controls were used to establish gating boundaries for every fluorochrome-conjugated antibody of the mixture. For bone marrow chimera experiments, CD45.2 was used to identify donor cells and immune cells were identified as follows: neutrophils ($CD45.2^+CD11b^{hi}Ly6G^{hi}$) and inflammatory monocytes ($CD45.2^+CD11b^{hi}Ly6G^{neg}F4/80^+CCR2^{hi}$). Data were acquired with a Special Order Research Product (SORP) flow cytometer (LSR II, BD) or an ARIA II (BD) and analyzed with FlowJo software (version 10.0.7, Tree Star).

Quantitative Real-Time RT-PCR. $CD4^+$ T cells from the spleens of WT and $Il1b^{-/-}$ mice were sorted based on their expression of CD45 and CD4 using a SORP flow cytometer (FACSAria II; BD Biosciences). Cells were centrifuged and homogenized using Qiazol Lysis Reagent (Qiagen). Total RNA was extracted using the miRNeasy Micro Kit On-column DNase (Qiagen), following the manufacturer's instructions. First-strand cDNA synthesis was accomplished using all of the RNA samples extracted above in a reaction containing 200 U of SuperScript IV RNase H-RT (Thermo Fisher Scientific), 150 ng of oligo-dT_{20}, 50 ng of random hexamers, 1× SSIV buffer, 500 μM deoxynucleotides triphosphate, 5 mM DTT, and 40 U of Protector RNase inhibitor (Roche Diagnostics) in a final volume of 20 μL. The reaction was incubated at 25 °C for 10 min and then at 50 °C for 20 min, followed by inactivation by incubation at 80 °C for 10 min. cDNA corresponding to 4% of the sample was used to perform fluorescent-based real-time PCR quantification using the LightCycler 480 (Roche Diagnostics). Reagent LightCycler 480 SYBRGreen I Master (Roche Diagnostics) was used as described by the manufacturer. The conditions for PCR reactions were as follows: 45 cycles, DMSO 2% denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s, elongation at 72 °C for 14 s, and reading for 5 s. A melting curve was generated to assess nonspecific signal. Relative quantity was calculated using the Delta Ct method. Normalization was performed using glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) as a reference gene. Primer sequences are given in Table S2. Primer pairs were designed using GeneTool 2.0 software (Biotools Inc.), and their specificity was verified by blast in the GenBank database. Primers were purchased from IDT (Integrated DNA Technology).

Stimulation of MOG-Reactive $CD4^+$ T Cells. Spleens from T-cell donors ($2D2^{+/-}$ or $2D2^{+/-}$::$Il1b^{-/-}$ mice) were harvested and processed as described before. CD4 T cells were isolated using a magnetic separation kit from Miltenyi Biotec. T cells were labeled using the CellTrace Violet dye (Thermo Fisher Scientific), according to the manufacturer's instructions, and washed twice in PBS before use. For APCs, spleens were isolated from EAE mice (C57BL/6 or $Il1b^{-/-}$) 7 d after immunization. CD4 T cells were depleted using the Miltenyi Biotec magnetic separation kit, and the cell suspension was irradiated with a dose of 20 Gy. $CD4^+$ T cells and APCs were resuspended in DMEM (Thermo Fisher Scientific) + 10% FBS and plated at a ratio of 5:1 (APCs:$CD4^+$ T cells) in round-bottom culture plates for 4 d (37 °C, 5% CO_2). $MOG_{35–55}$ was added to the medium (final concentration of 0.1, 1, or 10 μg/mL) or replaced with saline (0). Where indicated, anakinra (100 μg/mL; Swedish Orphan Biovitrum) or a mouse anti-mouse IL-1β neutralizing antibody (10 μg/mL; Novartis Pharma AG) was added to the medium. Secreted IFNγ and IL-17 were measured in the supernatants using ELISA according to the manufacturer's instructions (R&D Systems). For the generation of conditioned media, $CD11b^+$ cells and $CD4^+$ T cells were magnetically sorted (Myltenyi Biotec) and cultured in UltraCULTURE media (Lonza) without serum.

Immunostaining and Histology. Anesthetized mice were transcardially perfused with ice-cold PBS followed by 1% PFA (pH 7.4) before their spinal cords were removed. Tissues were postfixed for 48 h and transferred into a 20% sucrose solution for 24 h. Spinal cords were embedded in Shandon M-1 Embedding Matrix (Thermo Fisher Scientific) and 14 μm-thick coronal sections cut using a cryostat (Leica Biosystems). Sections were collected directly onto Surgipath X-tra microslides (Leica Biosystems) and stored at −20 °C until use. Primary antibodies used for immunostaining and dilutions are available in Table S1. Alexa Fluor 488, 555, or 633-conjugated antibodies (1:250 dilution; Thermo Fisher Scientific) were used as secondary antibodies and DAPI (Thermo Fisher Scientific) as a nuclear counterstain. Images were acquired on an inverted confocal microscope system (IX81; Olympus) equipped with Diode 405, Ar 488, HeNe1 543, and HeNe2 633 laser lines (Olympus).

Mouse BMECs and Monocyte Cultures and Myeloid Cell Transmigration Assay. Primary cultures of mouse BMECs were established using brains harvested from adult C57BL/6 or $IL\text{-}1R1^{-/-}$ mice, as previously described (5, 17). Briefly, BMECs were cultured in DMEM supplemented with 20% FBS, 1 ng/mL basic fibroblast growth factor, 100 μg/mL heparin, and 1.4 μM hydrocortisone with antibiotics and antimycotics. Medium was supplemented with puromycin (10 μg/mL) for the first 2 d of culture, after which the puromycin concentration was adjusted to 4 μg/mL. Fifty thousand BMECs were plated on 3-μm pore-sized gelatin-coated Boyden chambers (Corning) and allowed to reach confluence (4 d) before being used.

For the transmigration assays, bone marrow from C57BL/6 or $Il1b^{-/-}$ mice was collected as described above. Neutrophils ($Ly6G^+$), monocytes ($Ly6G^{neg}Gr1^+$), or total $Gr1^+$ cells were magnetically sorted using the Myeloid-Derived Suppressor Cell Isolation Kit from Miltenyi Biotec. A total of 10^6 cells were placed in the upper reservoir of Boyden chambers, and cells were allowed to transmigrate for 18 h. Anakinra (100 μg/mL) or an anti–IL-1β neutralizing antibody (10 μg/mL) was added to the upper and bottom chamber at the same time. Cell number in the bottom chamber was manually counted.

Primary monocytes were cultured according to the method published by Francke et al. (40). Briefly, bone marrow cells (10^6 cells per milliliter) were cultured for 5 d in RPMI containing FBS (10%), M-CSF (20 ng/mL), and antibiotics/antimycotics on ultra-low adherence plates. Fresh medium was added to the culture on day 3. Nonadhering monocytes were harvested on day 5, placed in 24-well plates, and stimulated for 24 h with conditioned media from BMECs, either untreated or stimulated with IL-1β (10 ng/mL). GM-CSF neutralizing antibody (5 μg, clone MP1-22E9) was added at the same time. Monocytes were stained with a viability marker and antibodies against CD45, CD11b, Ly6C, CD11c, CD80, CD86, and MHCII (Table S1).

Human BMEC Culture. Primary cultures of human BMECs were prepared from temporal lobe tissue obtained during surgical resection in patients suffering from epilepsy, as previously described (5). BMECs were grown in medium composed of M199 (Thermo Fisher Scientific) supplemented with 10% FBS, 5% normal human serum, 1.95 μg/mL EC growth supplement, and insulin–transferrin–selenium premix on 0.5% gelatin-coated tissue culture plates (all reagents from Sigma-Aldrich unless otherwise indicated). BMECs were stimulated for 18 h with 10 ng/mL of human recombinant IL-1β (R&D Systems).

2P-IVM. 2P-IVM was performed in LysM-eGFP and LysM-eGFP::$Il1b^{-/-}$ at 12 d.p.i. (average day of EAE onset in LysM-eGFP mice). Animals were anesthetized with 1–2% isoflurane (vol/vol) and a small laminectomy performed at the T12-L1 vertebral level to expose the lumbar spinal cord. The dura mater was removed using the tip of a 27-gauge needle and the spinal cord kept moist afterward using sterile HBSS without Ca^{2+}/Mg^{2+} (Life Technologies). Mice were then transferred to a custom-made stabilization device adapted for 2P-IVM (described in ref. 41) and body temperature maintained at 37 °C using a temperature controlling device (RWD Life Science Co.). Labeling of $CCR2^+$ inflammatory monocytes was achieved through tail vein injection of rat anti–CCR2-PE (2.5 μg; R&D Systems) 10 min before imaging, whereas blood vessels were labeled using Qdot 705 (Qtracker 705, 1% wt/vol in saline; Life Technologies). The lumbar spinal cord region to image was next placed under the 25× objective and Gel-Seal (GE Healthcare) carefully applied on the bone surrounding the laminectomy to create a watertight

cavity. All images were acquired on an Olympus FV1000 two-photon microscope, and the MaiTai DeepSee laser (Spectra-Physics, Newport Corp.) was tuned at a wavelength of 925 nm to simultaneously visualize eGFP (LysM), PE (CCR2), and Qdot 705. Time-lapse videos were processed with the Intravital_Microscopy_Toolbox to remove motion artifacts (41).

Primary Cortical Neuron Culture. Dissociated cortical neurons were prepared from embryonic day 16–17 C57BL/6 mice. Cerebral cortices were dissected, digested with 0.25% trypsin-EDTA, mechanically dissociated, and cultured for 24 h on dishes coated with 50 μg/mL Poly-L-Lysine (Sigma-Aldrich). Neurons were grown in Neurobasal media (Gibco) supplemented with 2% B27 (Gibco), 1% N2 (Gibco), 1% penicillin/streptomycin (Gibco), and 1% L-glutamine (Gibco). Treated neurons were fixed, stained with an anti–β-III tubulin antibody, and Hoescht nuclear stain. Automated image acquisition was performed using an ImageXpress system, and analysis was performed by the Neurite Outgrowth module of the MetaXpress software (Molecular Devices).

Statistical Analysis. All data are shown as the mean ± SEM. Statistical evaluations were performed with one- or two-way ANOVA where appropriate. Specifically, one-way ANOVA test was used when only one factor was affecting the results, such as genotype or culture treatment. Two-way ANOVA was used to evaluate the statistical significance of results when two independent variables affected the dataset, such as the effect of genotype and time/treatment. Post-ANOVA comparisons were made using the Bonferroni correction. All statistical analyses were performed using Prism 6 (GraphPad Software). A P value < 0.05 was considered statistically significant.

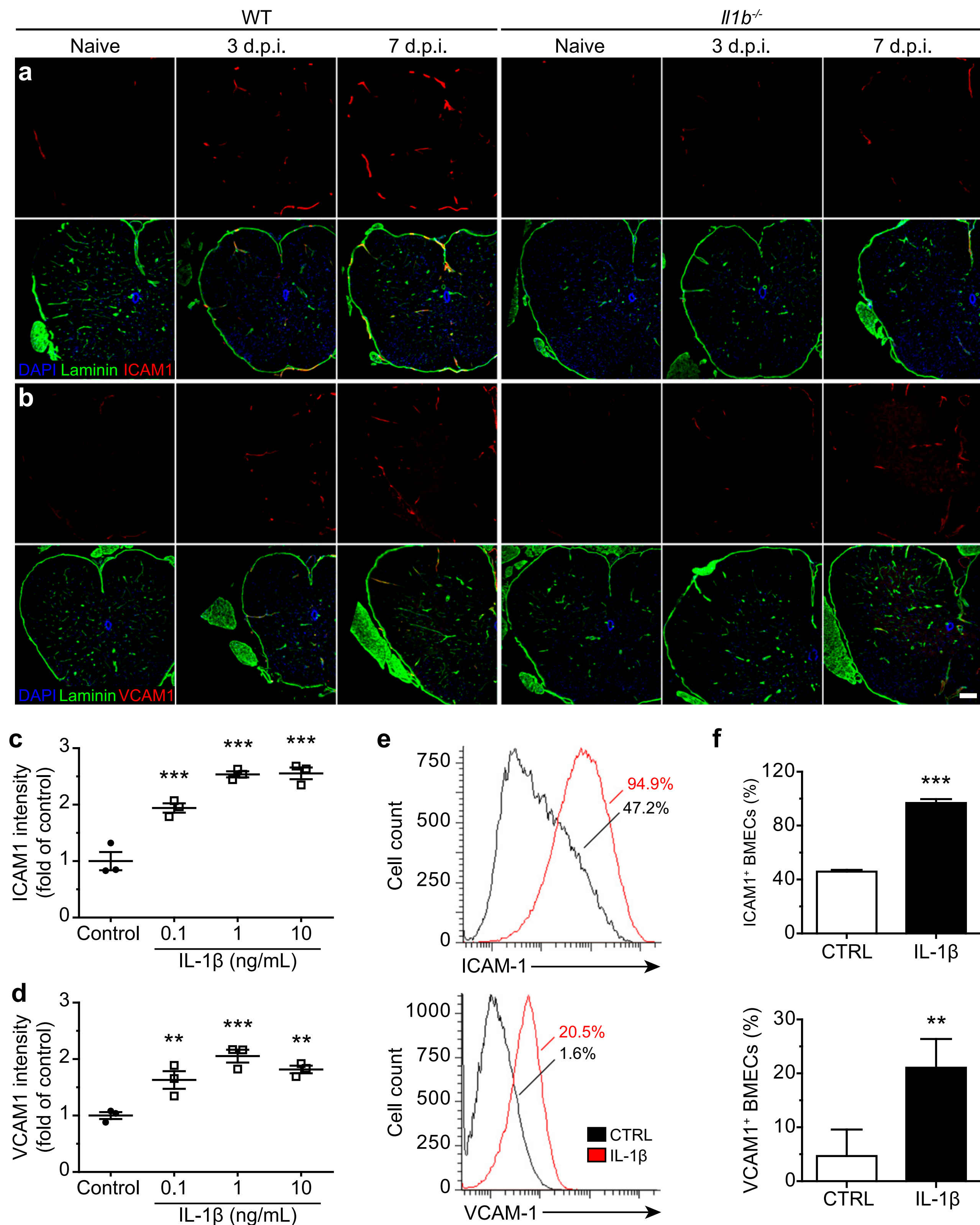


Fig. S1. Expression of ICAM-1 and VCAM-1 in spinal cord blood vessels of WT and *Il1b*$^{-/-}$ mice. (*A* and *B*) Immunofluorescence staining of the spinal cord of WT (*Left*) and *Il1b*$^{-/-}$ (*Right*) mice showing the expression of the cell adhesion molecules ICAM-1 (red, *A*) and VCAM-1 (red, *B*) in unimmunized (naive) mice and immunized (EAE) mice killed at 3 or 7 d.p.i. The basement membranes surrounding blood vessels were immunostained with an anti-laminin antibody (green) and sections counterstained with DAPI (blue). (*C* and *D*) Quantification of immunocytochemical signal intensities obtained for ICAM-1 (*C*) and VCAM-1 (*D*) following treatment of primary mouse BMECs with increasing concentrations of recombinant mouse IL-1β or saline (control) for 24 h. (*E*) Representative flow cytometry plots showing the expression of ICAM-1 (*Top*) and VCAM-1 (*Bottom*) by human BMECs after stimulation with recombinant human IL-1β (10 ng/mL) or saline (control, CTRL). (*F*) Quantification of the percentage of BMECs expressing ICAM-1 (*Top*) and VCAM-1 (*Bottom*) following 18 h of treatment with IL-1β. Data are expressed as mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$; (*C* and *D*) one-way ANOVA followed by a Bonferroni post hoc test ($n = 3$ per condition); (*F*) Student's *t* test ($n = 2$–5). [Scale bar, (*A* and *B*) 100 μm.]

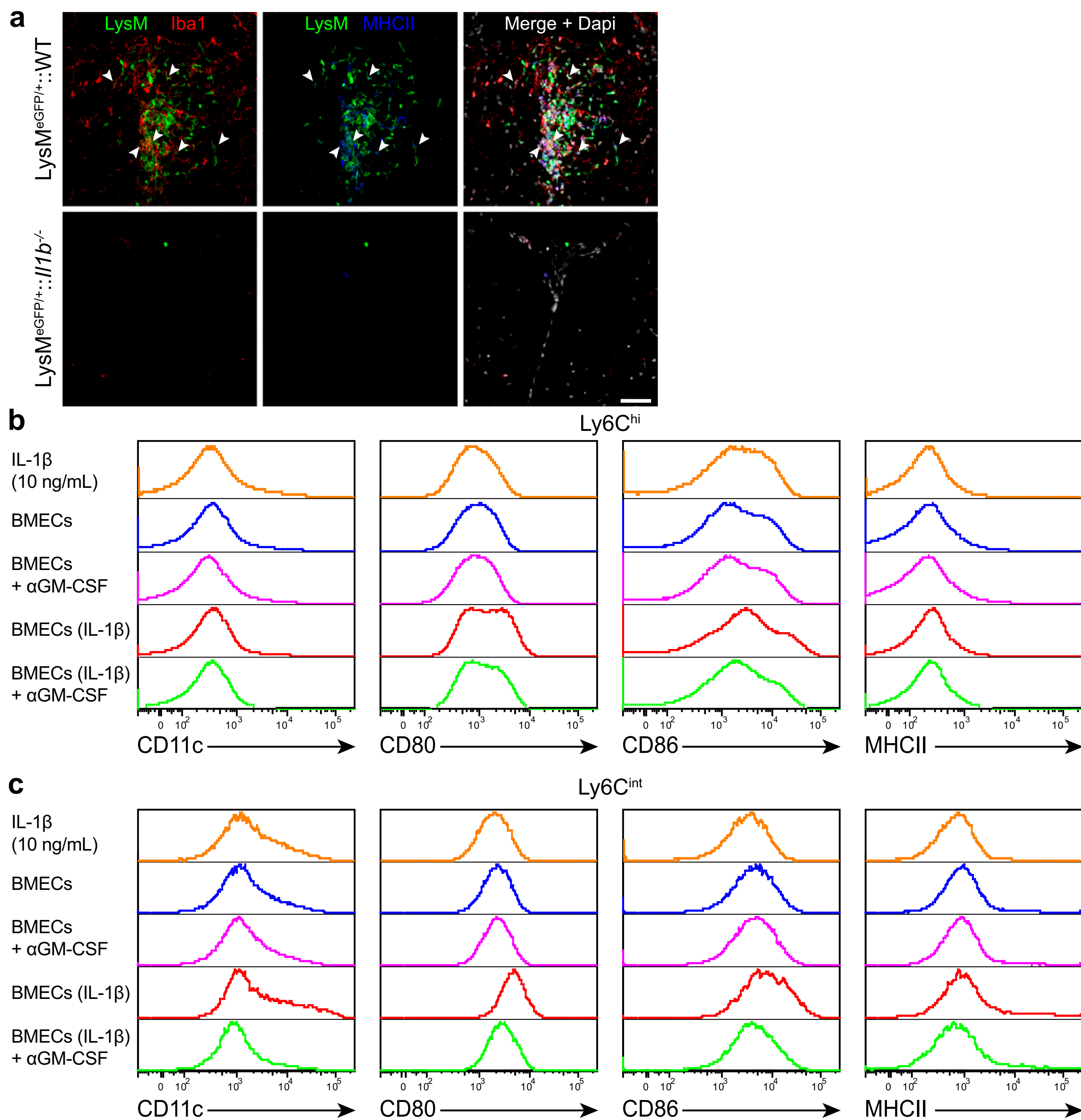


Fig. S2. Expression of antigen presentation-associated surface molecules by MDMs and monocytes. (*A*) Representative confocal images showing MDMs, identified by their coexpression of LysM-eGFP (green) and Iba1 (red), expressing MHCII (blue) in the spinal cord of WT EAE mice. White arrows point at LysM, Iba1, and MHCII triple-positive cells. DAPI (gray) was used as a nuclear counterstain. (*B* and *C*) Representative histograms showing the expression of CD11c, CD80, CD86, and MHCII on Ly6C^{hi} (*B*) and Ly6C^{int} (*C*) monocytes. Monocytes were treated for 24 h with IL-1β alone (10 ng/mL) or with conditioned medium collected from untreated or IL-1β–activated BMECs, supplemented or not with a GM-CSF neutralizing antibody (αGM-CSF). [Scale bar, 50 μm (*A*).]

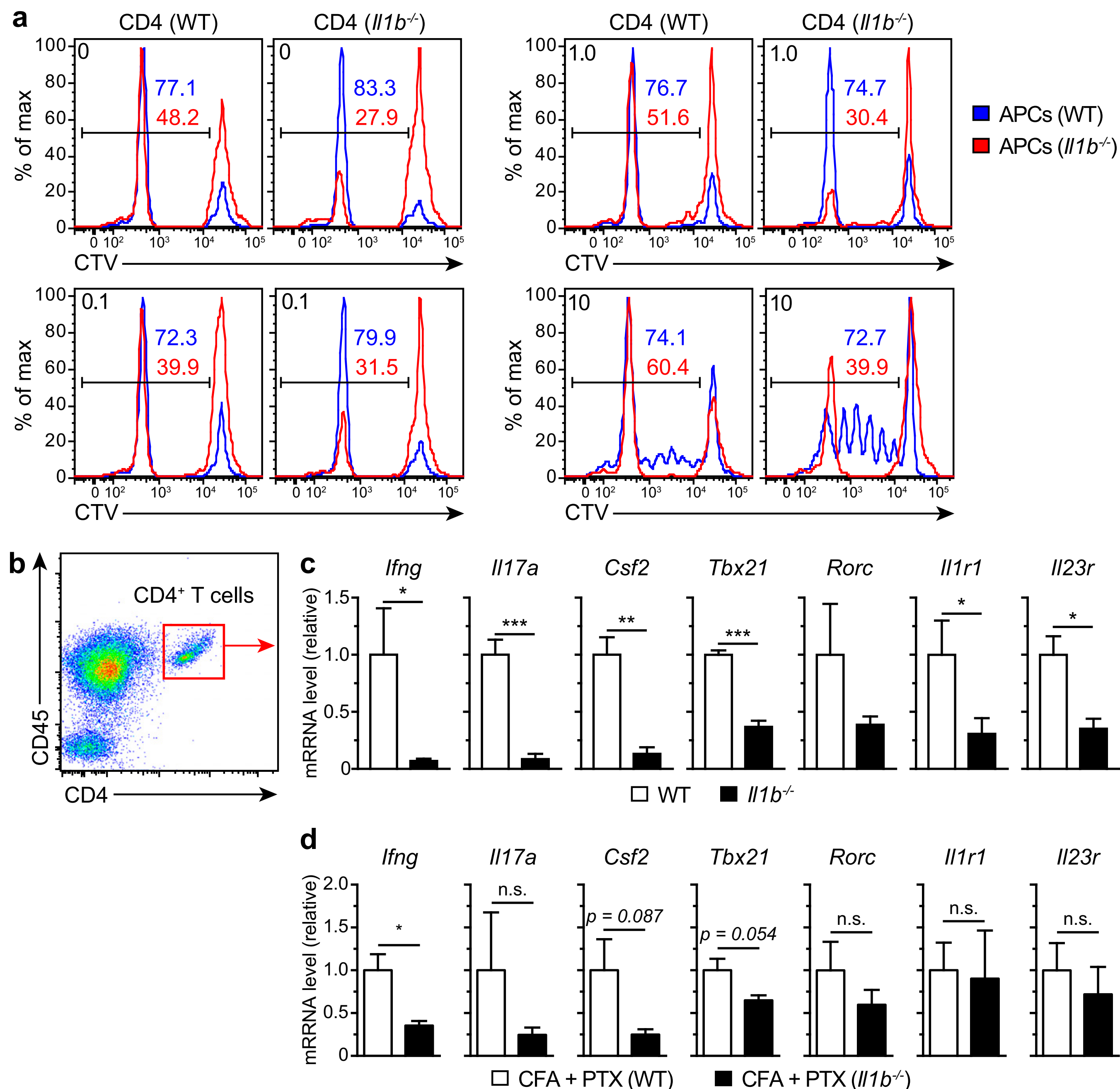


Fig. S3. CD4+ T-cell activation in the presence of APCs from *Il1b*−/− and WT mice. (*A*) Representative flow cytometry plots depicting the intensity level of the cell division marker Cell Trace Violet (CTV) in CD4+ T cells cocultured with APCs isolated from either WT (blue) or *Il1b*−/− (red) mice. $MOG_{35–55}$ concentrations (in μg/mL) are shown in the top left corners and the frequencies (%) of divided CD4+ T cells displayed in their respective color. (*B*) Representative dot plot of CD4+ T cells isolated from the spleen of a WT mouse at 7 d.p.i. (*C*) Relative mRNA expression levels of *Ifng*, *Il17a*, *Csf2*, *Tbx21*, *Rorc*, *Il1r1*, and *Il23r* in CD4+ T cells isolated from either WT or *Il1b*−/− mice. (*D*) Relative mRNA expression levels of *Ifng*, *Il17a*, *Csf2*, *Tbx21*, *Rorc*, *Il1r1*, and *Il23r* in CD4+ T cells isolated from either WT or *Il1b*−/− mice treated with only CFA and PTX. mRNA levels were normalized using *Gapdh* and are expressed as a ratio of the WT group (mean ± SEM). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; two-way ANOVA followed by a Bonferroni post hoc test; $n = 3–4$ per condition. Data are representative of two independent experiments.

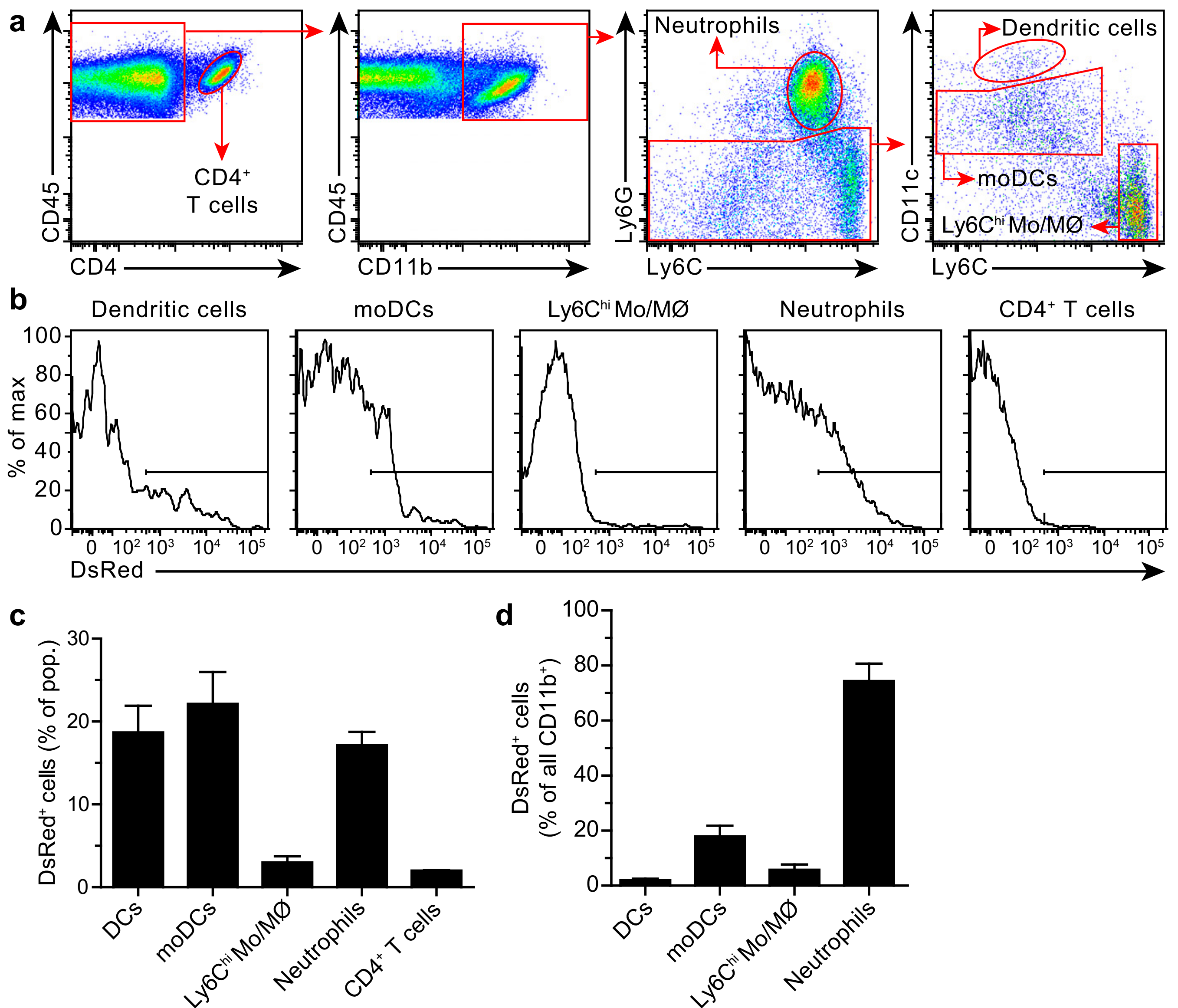


Fig. S4. IL-1β production by splenic APCs after EAE immunization. (*A*) Gating strategy used to identify immune cells producing pro–IL-1β in the spleen of pIL-1β–DsRed reporter mice at 7 d.p.i. (n = 3). (*B*) Representative flow cytometry plots showing the expression of DsRed (pro–IL-1β) in DCs, moDCs, $Ly6C^{hi}$ monocytes/macrophages (Mo/MØ), neutrophils, and $CD4^+$ T cells. (*C*) Frequency of $DsRed^+$ cells, expressed as a percentage of the population. (*D*) Frequency of $DsRed^+$ cells expressed as a percentage of the DsRed-expressing myeloid cells ($CD45^+CD11b^+$). Data are expressed as mean ± SEM.

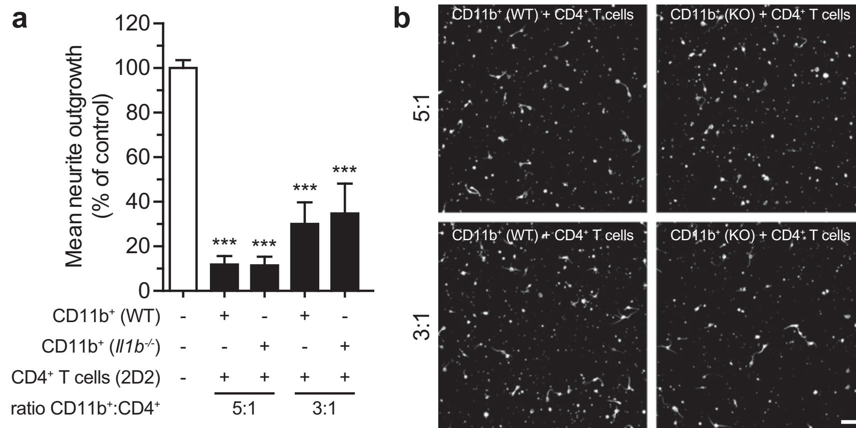


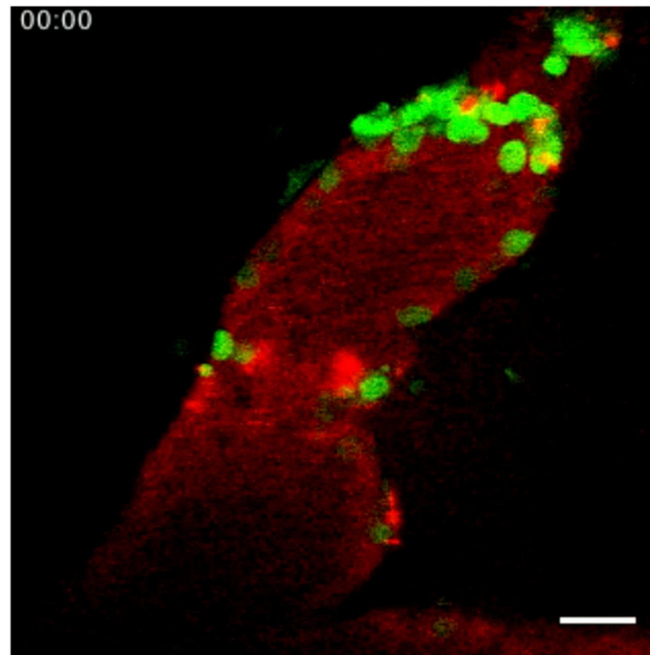
Fig. S5. Neurotoxicity of cocultures of CD11b^{+} myeloid cells and CD4^{+} T cells. (*A*) Quantification of the average neurite length normalized to the control condition (mean ± SEM). Cocultures were performed using either a 5:1 or 3:1 ratio of CD11b^{+} and CD4^{+} cells, as indicated. (*B*) Representative images of primary cortical neurons treated for 24 h with the indicated conditioned media and immunostained against β-tubulin (white signal). ****P* < 0.001 versus control; one-way ANOVA followed by a Bonferroni post hoc test (*n* = 3–7). [Scale bar, (*B*) 50 μm.]

Table S1. List of antibodies used in the present study

Antibody	Host	Clone	Supplier	Concentration
Flow cytometry				
CD45-PerCP	Rat	30-F11	BD Biosciences	1 μg per 10^6 cells
CD45.1-PE	Rat	A20	BD Biosciences	1 μg per 10^6 cells
CD45.2-PerCP-Cy5.5	Rat	104	BD Biosciences	1 μg per 10^6 cells
CD11b-Alexa 700	Rat	M1/70	BD Biosciences	1 μg per 10^6 cells
Ly6C-V450	Rat	AL-21	BD Biosciences	0.6 μg per 10^6 cells
Ly6G-PeCy7	Rat	1A8	BD Biosciences	1 μg per 10^6 cells
Ly6G-APC-Cy7	Rat	1A8	BD Biosciences	1 μg per 10^6 cells
CD115-APC	Rat	AFS98	eBioscience	1 μg per 10^6 cells
CD4-FITC	Rat	RM4-5	eBioscience	1 μg per 10^6 cells
CD4-PerCp-Cy5.5	Rat	RM4-5	eBioscience	1 μg per 10^6 cells
CD11c-APC	Armenian hamster	N418	BD Biosciences	1 μg per 10^6 cells
CD80-FITC	Rat	16–10A4	BD Biosciences	1 μg per 10^6 cells
CD86-PeCy7	Rat	GL1	BD Biosciences	1 μg per 10^6 cells
MHCII-PE	Rat	M5/114.15.2	eBioscience	1 μg per 10^6 cells
CCR2-PE	Rat	475301	R&D Systems	1 μg per 10^6 cells
IFNγ-FITC	Rat	XMG1.2	eBioscience	1 μg per 10^6 cells
IL-17A-PeCy7	Rat	TC11-18H10.1	BioLegend	1 μg per 10^6 cells
Immunostaining				
ICAM1	Rat	YN1/1.7.4	BioLegend	1/1,000
VCAM1	Rat	M/K-2	Abcam	1/1,000
MHCII	Rat	M5/114.15.2	BD Biosciences	1/2,000
CD86	Rat	GL1	BD Biosciences	1/50
GFP	Rabbit	Polyclonal	Thermo Fisher Scientific	1/500
Ki67	Mouse	B56	BD Biosciences	1/50
CD4	Rat	RM4-5	Cedarlane	1/750
Laminin	Rabbit	Polyclonal	Dako	1/1,000
β-tubulin	Mouse	TUJ1	BioLegend	1/1,000

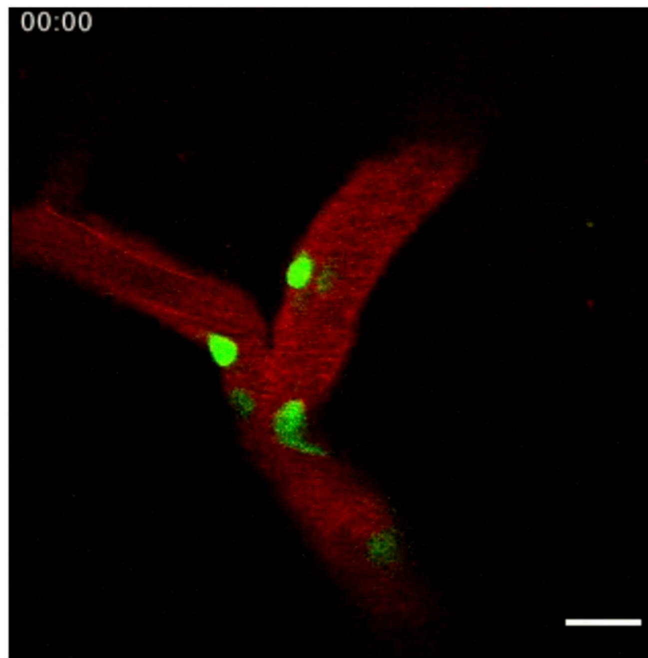
Table S2. List of qRT-PCR primers used in this study (see Fig. S3)

GenBank ID	Gene	Forward primer, 5′–3′	Reverse primer, 5′–3′
NM_009969	*Csf2*	ACTTTTCCTGGGCATTGTGGTCTAC	GGGTCTGCACACATGTTAGCTTCT
NM_010552	*Il17a*	ATCCCTCAAAGCTCAGCGTGT	AGGGTCTTCATTGCGGTGGAG
NM_008337	*Ifng*	GGAGGAACTGGCAAAAGGATG	GACCTCAAACTTGGCAATACTCATG
NM_011281	*Rorc*	TAGCACTGACGGCCAACTTACTCT	CTCGGAAGGACTTGCAGACATT
NM_019507	*Tbx21*	ACCGCTTATATGTCCACCCAGA	CGGGGCTGGTACTTGTGGAGA
NM_001123382	*Il1r1*	CACGGAGTCATTTGCTGGTCA	GCCGAGTGGTAAGTGTGTTGCT
NM_144548	*Il23r*	AGCACTGCCGACCAAGGAATC	AGGCATGAGGTTCCGAAAAGC
NM_008084	*Gapdh*	GGCTGCCCAGAACATCATCCCT	ATGCCTGCTTCACCACCTTCTTG



Movie S1. Myeloid cell dynamics in the spinal cord of a WT mouse with EAE. Representative 2P-IVM video of the spinal cord of a LysM-eGFP::WT mouse at 12 d.p.i. Neutrophils and inflammatory monocytes (green) are shown circulating, rolling, and adhering to the endothelium surface of a spinal cord blood vessel (red). (Scale bar, 20 μm.) Time is displayed as minutes:seconds.

Movie S1



Movie S2. Myeloid cell dynamics in the spinal cord of an *Il1b*$^{-/-}$ mouse with EAE. Representative 2P-IVM video of the spinal cord of a LysM-eGFP::*Il1b*$^{-/-}$ mouse at 12 d.p.i. Neutrophils and inflammatory monocytes (green) of immunized LysM-eGFP::*Il1b*$^{-/-}$ mice are capable of rolling and adhering to blood vessels (red), although to a lesser degree than in LysM-eGFP::WT mice with EAE. (Scale bar, 20 μm.) Time is displayed as minutes:seconds.

Movie S2