Supporting Information

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SI Materials and Methods

Generation of BMDCs. Mice were euthanized with CO_2. Fur, skin, and muscles were dissected from lower extremities of the animal (including tibia, femur, and hips). Bones were flushed using a 30-gauge × 12-mm needle and sterile cold PBS. The obtained cell suspension was passed through a 70-μm cell strainer and washed with cold PBS. A red blood cell lysis (2 min, room temperature; RT) was performed using 1 mL ACK lysis buffer. Bone marrow cells were plated out in a non-tissue culture-treated Petri dish with BMDC culture medium [10% heat inactivated FCS (F2442-6X500ML; Sigma-Aldrich), 1% penicillin/streptomycin (P/S; 15070-063; Life Technologies), 50 μM β-mercaptoethanol (21985023; Thermo Fisher Scientific), and 2.5 ng/mL recombinant mouse GM-CSF (576306; BioLegend) in RPMI-1640 (7001612; Life Technologies)]. A complete medium change was performed every second day.

Leukocyte Isolation from Spleen. Mice were euthanized by CO_2 inhalation. Spleens and lymph nodes were removed, dissected into small pieces, and digested for 30 min at 37 °C and 5% CO_2 with collagenase D (0.4 mg/mL; 11088866001; Sigma-Aldrich) and DNase (20 μg/mL; 79254; Qiagen) in PBS. Digestion was terminated with 10 mM EDTA (ED-500G; Sigma-Aldrich) during the final 5 min at 37 °C and 5% CO_2. The digested tissue was passed through a 70-μm cell strainer to obtain single-cell suspensions. After a washing step with cold PBS, red blood cell lysis (2 min, RT) was performed using 1 mL ACK lysis buffer (A1049201; Thermo Fisher Scientific) per spleen. Cell concentration was determined, and samples were kept on ice until further processing.

Leukocyte Isolation from CNS. Mice were perfused with ice-cold PBS, the CNS was removed, dissected into small pieces, suspended in digest buffer (collagenase D 0.2 mg/mL and DNase 20 μg/mL in PBS; 4 mL per CNS), and incubated for 40 min at 37 °C and 5% CO_2. The digest was terminated with 10 mM EDTA during the last 5 min of incubation. The digested tissue was passed through a 70-μm nylon mesh. Pelleted cells were suspended in 30% Percoll (17-0891-01; GE Healthcare) in PBS and ultracentrifuged (Sorvall RC 6 Plus Superspeed Centrifuge; Thermo Fisher Scientific) for 30 min at 4 °C. The resulting lipid layer was sucked off and discarded. The remaining cell layer was transferred to fresh tubes and centrifuged at 400 × *g* and 4 °C for 5 min. Cell concentration was determined and samples were kept on ice until further processing.

Antibodies. PE-Cy7–conjugated anti-CD11c (clone: N418, 1:400; 117318; BioLegend), APC-conjugated anti-mouse I-A^b (clone: M5/114.15.2, 1:800; 107614; BioLegend), PerCP-Cy5.5– and biotin-conjugated anti-Ly6C (clone: HK1.4, 1:200; 128012 and 128004; BioLegend), APC-Cy7–conjugated anti-CD11b (clone: M1/70, 1:200; 101226; BioLegend), Pacific Blue-conjugated anti-CD19 (clone: 6D5, 1:400; 115523; BioLegend), Alexa Fluor 700-conjugated anti-CD19 (clone: eBio1D3, 1:200; 56-0193-82; eBioscience), Pacific Blue-conjugated anti-CD45.2 (clone: 104, 1:400; 109820; BioLegend), APC-Cy7–conjugated anti-CD45 (clone: 30-F11, 1:400; 557659; BD Biosciences), PE-conjugated anti-CD45.1 (clone: A.20, 1:200; 110707; BioLegend), Alexa Fluor 700-conjugated anti-Ly6G (clone: 1A8, 1:200; 127622; BioLegend), Brilliant Violet 650-conjugated anti-CD86 (clone: GL-1, 1:200; 105035; BioLegend), PE-Cy–conjugated anti-CD40 (clone: 3/23, 1:50; 124622; BioLegend), Alexa Fluor 700-conjugated anti-CD4 (clone: GK1.5, 1:200; 56-0041-82; eBioscience), APC-conjugated anti-CD4 (clone: GK1.5, 1:200; 100412; BioLegend), Brilliant Violet 785-conjugated anti-CD8α (clone: 53-6.7, 1:100; 100750; BioLegend), PE-CF594–conjugated anti-CD8α (clone: 53-6.7, 1:400; 562283; BD Biosciences), PerCP-Cy5.5–conjugated anti-CD44 (clone: IM7, 1:100; 45-0441-82; eBioscience), PE-conjugated anti–Siglec-H (clone: eBio440c, 1:800; 12-0333-82; eBioscience), Brilliant Violet 605-conjugated anti-CD25 (clone: PC61, 1:200; 102036; BioLegend), PerCP-Cy5.5–conjugated anti-Foxp3 (clone: FJK-16s, 1:100; 45-5773-82; eBioscience), unconjugated anti-CD3ε (clone: 145-2C11, 5 μg/mL; 553058; BD Biosciences), unconjugated anti-CD28 (clone: 37.51, 5 μg/mL; 553295; BD Biosciences), unconjugated anti–ATG5–ATG12 complex (1:400; WA-ABD10857.100; Biomol), unconjugated anti–ATG5–ATG12 complex (1:500; NB110-53818; Novus), unconjugated anti-LC3 (1:100; PM036; MBL), HRP-conjugated goat anti-mouse IgG (1:10,000; 172-1011-MSDS; Bio-Rad), HRP-conjugated β-actin (clone: 13G11; 1:50,000; 137402.100; Biomol), Alexa Fluor 488-conjugated rabbit anti-mouse IgG (H+L) (A-11059; Invitrogen).

Magnetic Activated Cell Sorting. All magnetic activated cell sorting (MACS) procedures were carried out using magnetic MicroBeads from Miltenyi (anti-CD11c, 130-097-059; anti-CD4, 130-049-201; anti-biotin, 120-000-900) and the autoMACS Pro Separator (130-092-545; Miltenyi) and in accordance with the provider's protocol recommendations.

Fluorescence Activated Cell Sorting. For some experiments (Fig. 5), MACS-derived $CD11c^+$-enriched populations were further purified using fluorescence activated cell sorting with a BD FACSAria III. Briefly, post-MACS $CD11c^+$-enriched cell suspensions were stained with fluorochrome-labeled antibodies (Pacific Blue-conjugated anti-CD45.2, clone: 104, 1:400; BioLegend, 109820; PE-Cy7–conjugated anti-CD11c, clone: N418, 1:400; BioLegend, 117318; APC-conjugated anti–I-A^b, clone: M5/114.15.2, 1:800; BioLegend, 107614) for 20 min on ice and in the dark. After a washing step, cells were resuspended in sorting buffer (2% BSA in PBS). Live $CD45^+CD11c^{hi}MHCII^{hi}$ single cells were determined as the target population and bulk-sorted into 15-mL tubes already containing 5 mL of the appropriate culture medium.

Flow Cytometry. For surface protein expression analysis, single-cell suspensions were incubated with Fc receptor block (22.4 μg/mL, 20 min, 4 °C; clone: 2.4G2, CUS-HB-197; Bio X Cell) followed by staining with the LIVE/DEAD Fixable Aqua Dead Stain Kit (L34957; Thermo Fisher Scientific) in PBS at 4 °C in the dark. Samples were washed twice in cold PBS followed by incubation with the respective fluorochrome-labeled antibodies in FACS buffer (0.5% BSA and 0.01% NaN_3 in PBS) for 30 min at 4 °C in the dark. During some (Fig. 3 and Fig. S2) experiments, intracellular cytokines were determined in CNS-derived and splenic $CD4^+$ T cells upon ex vivo restimulation with either $MOG_{35–55}$ peptide, $OVA_{323–339}$ peptide (ISQAVHAAHAEINEAGR; InvivoGen, vac-isq), or phorbol 12-myristate 13-acetate (PMA)/ionomycin (PMA: 1:50,000, Sigma-Aldrich, P1585; ionomycin: 1:747, Sigma-Aldrich, I3909) in R10 (10% FCS in RPMI-1640) supplemented with 1% P/S. Leukocytes were isolated and purified from the respective organs. For each animal, the organ-specific (CNS or spleen) cell suspension was divided into two groups determined by the restimulation agent ($MOG_{35–55}$ peptide,

$OVA_{323–339}$ peptide). For determination of cytokine production in naïve mice, splenocytes were restimulated with PMA/ionomycin. Brefeldin A (10 μg/mL; B5936-200UL; Sigma-Aldrich) was added to inhibit cytokine secretion by induction of retrograde transport from the Golgi apparatus back to the endoplasmic reticulum. Samples were incubated in restimulation media at 37 °C and 5% CO_2 for 4 h, and then washed twice in cold PBS followed by centrifugation at 400 × *g* and 4 °C for 5 min. After surface staining, cell pellets were resuspended in 200 μL of Cytofix/Cytoperm buffer (554714; BD Biosciences) and incubated for 30 min at 4 °C in the dark. After a washing step with 1× permeabilization/wash buffer (554714; BD Biosciences), cell pellets were resuspended in 50 μL staining solution containing the appropriate dilutions of antibodies against the proinflammatory cytokines of interest (FITC-conjugated anti–IFNγ, clone: XMG1.2, 1:400, BioLegend, 505806; PE-Cy7–conjugated anti–IL-17A, clone: TC11/18H10, 1:200, BioLegend, 506922; PE-conjugated anti–GM-CSF, clone: MP1-22E9, 1:250, BD Biosciences, 554406) in 1× permeabilization/wash buffer. Samples were incubated in the dark at 4 °C for 30 min. After two final washing steps in 1× permeabilization/wash buffer, cell pellets were resuspended in PBS and immediately recorded with the BD LSRFortessa using FACSDiva software v6.1.3 (BD Biosciences). Analysis was performed using the FlowJo software v9.3.1 (Tree Star).

Cell Lysis for Protein Quantification. The culture medium of the cell population of interest was removed, and cells were washed twice with cold PBS. Adherent cells were removed from the culture plate by trypsinization or gentle mechanical detachment using a cell scraper. Cell suspensions were washed and cell pellets were resuspended in an appropriate amount of freshly prepared cold lysis buffer (1% Nonidet P-40; 127087-87-0; Sigma-Aldrich) in PBS supplemented with 1× protease inhibitor mixture (11697498001; 50 μL per 1×10^6 cells; Sigma-Aldrich). Samples were incubated in lysis buffer for 30 min on ice and centrifuged at maximum speed and 4 °C for 10 min. The supernatant was transferred to a fresh 1.5-mL tube. At this stage, a small amount of each sample was set aside to use for determination of protein concentration. The remainder of each sample was stored at −80 °C until further use.

SDS/PAGE and Western Blot. For determination of protein concentrations in whole-cell lysates, the Pierce BCA Protein Assay Kit (23225; Thermo Fisher Scientific) was used according to the manufacturer's recommendations. Protein samples were diluted with 4× Laemmli buffer (11697498001; Bio-Rad) and boiled for 5 min at 95 °C. The 0.75-mm gels were assembled in a Bio-Rad stacking system, and protein samples including a protein ladder (1610374; Bio-Rad) were loaded into the gel pockets. Gels were run at 70 V until samples had entered the resolving gel, when the voltage was increased to 100 V until the protein front had almost reached the bottom of the gel. A polyvinylidenefluoride (PVDF) membrane (GE Healthcare; RPN303F) was activated in 100% methanol (67-56-1; Sigma-Aldrich) for 2 min. Appropriately tailored Whatman filter papers (588-3186; VWR International), PVDF membrane, and gel were soaked in 1× transfer buffer (25 mM Tris base, Sigma-Aldrich, 77-86-1; 192 mM glycine, Sigma-Aldrich, 56-40-6; 20% methanol) for equilibration for 10 min. Proteins were transferred via a semidry transfer system at 10 V for 1 h. The membrane was removed and blocked for 1 h at RT in blocking solution [4% skimmed milk and 0.1% Tween 20 (9005-64-5; Sigma-Aldrich) in PBS] followed by incubation with primary antibody diluted in blocking solution overnight at 4 °C on a shaker. After washing for at least 5 min with PBS-T (0.1% Tween 20 in PBS), the membrane was incubated for 1 h at RT with secondary antibody diluted in blocking solution on a shaker. The membrane was again washed three times for at least 5 min with PBS-T and finally once with PBS for 5 min. Pierce ECL Western Blotting Substrate (32106; Thermo Fisher Scientific) was used to develop the membrane. Protein bands were visualized with a Vilber Fusion FX detector.

Carboxyfluorescein Diacetate Succinimidyl Ester Labeling/CFSE Dilution. Single-cell suspensions of MACS-purified $CD4^+$ T cells were prepared in 0.1% BSA in PBS at a final concentration of 5×10^7 cells per mL. Carboxyfluorescein diacetate succinimidyl ester (CFDA-SE; 65-0850-84; eBioscience) was added to the cells at a final concentration of 1 μM and incubated for 10 min at 37 °C. Cells were quenched by adding cold R10 followed by a 5-min incubation on ice. Afterward, cells were washed three times with R10.

DC:T Cell Coculture. MACS-purified $CD4^+$ T cells were labeled with CFDA-SE and cocultured with FAC-sorted splenic DCs in R10 supplemented with 50 μM β-mercaptoethanol and 1% P/S. Coculture was performed in a 96-well U-bottom plate at a 1:3 cell ratio (DC:T cells). Either anti-CD3/anti-CD28 antibodies, $MOG_{35–55}$ peptide, uncoated beads, beads coated with Pam_3CSK_4 (tlrl-pms; InvivoGen), or beads coated with $MOG_{1–125}$ protein (at different bead:DC ratios) were added to the wells. All conditions were incubated for 4 d at 37 °C and 5% CO_2. To assess CFSE dilution, cultured cells were stained with APC-conjugated anti-CD4 (clone: GK1.5, 1:200; 100412; BioLegend), and the LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (L10119; Thermo Fisher Scientific) was used for dead-cell exclusion. CFSE dilution was recorded on a BD FACSCanto II using FACSDiva v6.1.3 software (BD Biosciences) and analyzed with FlowJo software v9.3.1 (Tree Star).

In Vivo Priming Assay. On day 0, DC-*Atg5*$^{-/-}$ and DC-*Atg5*$^{+/+}$ mice were immunized with 100 μg MOG protein or OVA protein (vac-pova; InvivoGen) in CFA. Additionally, animals received 200 ng pertussis toxin i.p. on days 0 and 2. Animals were euthanized on day 7, and splenic $CD4^+$ T cells were purified via MACS separation and stained with CFDA-SE. As a negative control, splenic $CD4^+$ T cells isolated from nonprimed naïve animals were used. In parallel, splenic $CD11c^+$ cells from naïve C57BL/6 wild-type mice were purified and pulsed with either $MOG_{35–55}$ or $OVA_{323–339}$ peptide (both 20 μg/mL). $CD4^+$ T cells (6×10^5) were cocultured with increasing amounts of peptide-pulsed $CD11c^+$ wild-type DCs in 96-well U-bottom plates in a total volume of 200 μL of R10 supplemented with 1% P/S and IL-2 (10 ng/mL), GM-CSF (2.5 ng/mL), and 50 μM β-mercaptoethanol. All conditions were performed in triplicate. After 4 d of coculture, cell-culture supernatants were collected for analysis of IFNγ concentrations by ELISA. $CD4^+$ T cells were analyzed for CFSE dilution on a BD FACSCanto II using FACSDiva v6.1.3 software (BD Biosciences) and analyzed with FlowJo software v9.3.1 (Tree Star).

Coating of Polystyrene Beads with $MOG_{1–125}$ Protein. Polystyrene beads were coated with 50 μg of $MOG_{1–125}$ protein per 1×10^8 beads. Beads (1×10^8) were prewashed with 1 mL PBS and then centrifuged at 600 × *g* for 5 min. The washing step was repeated and beads were resuspended in 950 μL PBS. Beads were then added to 50 μL $MOG_{1–125}$ protein (stock 1 mg/mL) and mixed by pipetting. The coating was performed for 2 h at RT. Afterward, beads were washed five times with PBS and counted.

Phagocytosis Assay. Preparation of cells for immunofluorescence microscopy was as follows: BMDCs were generated as described above. Between days 7 and 10 of culture, BMDCs were seeded in eight-well chamber slides. BMDCs (7.5×10^4) were plated into each well and cells were kept at 37 °C and 5% CO_2 overnight in

order for the cells to settle. In parallel, the polystyrene beads were coated with $MOG_{1–125}$ protein as described above. On the next day, BMDCs were cultured under the indicated conditions for 4 h at 37 °C and 5% CO_2. After incubation of BMDCs with $MOG_{1–125}$ protein-coated beads, immunofluorescence microscopy was performed.

Cell Culture. Cells were treated in a sterile biological safety hood and cultured in an incubator at 37 °C, 5% CO_2 with the specific medium containing the appropriate nutritional and survival factors. MOG-overexpressing and wild-type MO3.13 cells (65) (oligodendroglial cells; WT ODC and ODC^{MOG+}) were cultured in DMEM supplemented with 1% P/S and 10% FCS. RAW 264.7 cells were cultured in RPMI supplemented with 1% P/S and 10% FCS.

Purification of Anti-MOG (8-18C5) Antibody. The anti-MOG (8-18C5) antibody was used to confirm MOG expression of the ODC^{MOG+} cell line. The antibody was obtained by cloning of the 8-18C5 hybridoma-derived *Igh* and *Igk* variable-region sequences into human IgG1 heavy-chain and κ light-chain expression vectors as described elsewhere (66). The antibody was purified by cotransfecting the calcium phosphate-precipitated expression vectors in HKB-11 cells. The medium was exchanged to DMEM (11995065; Thermo Fisher Scientific) containing 1% P/S and 1% Nutridoma-SP (11011375001; Sigma-Aldrich) after 12 h. Culture supernatant was harvested 4 d later, and antibodies were purified using HiTrap Protein G HP columns (GE Healthcare) according to the manufacturer's instructions using GE Aekta Prime Plus.

UVB Irradiation. ODC^{MOG+} were UVB-irradiated at 870 mJ/cm^2 using a Waldmann UV 181 BL irradiation unit. On day 0, cells were detached, washed in PBS, and plated in tissue-culture dishes and cultured in serum-free DMEM supplemented with 1% P/S overnight at 37 °C, 5% CO_2 (1×10^5 cells per dish, 2-mL volume). On day 1, medium was removed and 2 mL of PBS was added to each dish. Cells were UVB-irradiated and, after irradiation, PBS was substituted with fresh serum-free medium. Cells were again incubated overnight at 37 °C, 5% CO_2. Surface Ptd-L-Ser^+ of irradiated (Ptd-L-Ser^{hi} ODC^{MOG+}) and non-irradiated MO3.13 cells (Ptd-L-Ser^{lo} ODC^{MOG+}) were quantified 1 d after irradiation by flow cytometry analysis using annexin V staining.

Quantification of Ptd-L-Ser^+ Cells. Single-ODC^{MOG+} cell suspension was washed twice with cold PBS and the pellet was resuspended in 1× annexin V binding buffer (422201; BioLegend) at a concentration of 1×10^6 cells per mL. FITC-conjugated annexin V (1:20; 556420; BD Biosciences) was added, and cells were incubated for 15 min at RT in the dark. Suspensions were washed and resuspended in 400 μL of annexin V staining buffer. Samples were acquired on a BD FACSCanto II within 1 h using FACSDiva v6.1.3 software (BD Biosciences) and analyzed with FlowJo software v9.3.1 (Tree Star).

Neriifolin in Vivo Treatment. Animals were injected i.p. with neriifolin (Sigma; S961825; 0.25 mg/kg diluted in 0.5% ethanol/PBS) or vehicle control (0.5% ethanol/PBS) 1 d before active immunization or adoptive transfer of donor cells. Injections were repeated every second day for eight injections total.

Quantification of LC3-Decorated Phagosomes. RAW 264.7 cells were plated out in 0.01% poly-L-lysine (P4707; Sigma-Aldrich) pretreated eight-well glass chamber slides (NUNC-177402; Nunc Lab-Tek) at 2×10^5 per chamber. Cells were incubated with 1 μM neriifolin or vehicle control (0.5% ethanol in PBS) for 6 h. Alexa Fluor 488-labeled zymosan (6×10^5; Z23373; Life Technologies) or the same amount of inert beads (CLB9; Sigma-Aldrich) was added for 20, 40, 60, and 80 min. After the indicated incubation time, cells were fixed, permeabilized, and stained with anti-LC3 and DAPI. The amount of zymosan-containing $LC3^+$ LAPosomes was counted in 10 frames per time point and condition.

Immunocytochemistry. Cells were fixed with 3% paraformaldehyde in PBS (CAS 3052-89-4; Santa Cruz Biotechnology) for 15 min and then washed twice with PBS. Cells were permeabilized with 0.1% Triton-X (X100-500ML; Sigma-Aldrich) in PBS and then blocked for 30 min at RT [1% BSA, 10% normal goat serum (NGS; G9023-5ML; Sigma-Aldrich) in PBS]. Afterward, cells were incubated with primary antibody (rabbit anti-LC3; PM036; MBL International) in 0.1% saponin (47036-50G-F; Sigma-Aldrich), 10% NGS in PBS for 1 h at RT. Chambers were washed twice with 0.1% saponin in PBS followed by a 45-min incubation with secondary antibody in 0.1% saponin, 10% NGS in PBS (Alexa Fluor 555-conjugated anti-rabbit; A-11070; Thermo Fisher Scientific). The cell nucleus was counterstained with DAPI (1:5,000; D1306; Thermo Fisher Scientific) for 2 min. The slides were mounted with ProLong Gold Antifade Reagent (P36930; Thermo Fisher Scientific) and topped with a cover slide (24 × 50 mm; no. 1.5 thickness: 0.16 to 0.19 mm). Slides were let sit for 24 h at RT in the dark, and then kept at 4 °C for long-term storage. Pictures were acquired using a 63×, 1.4 N.A. oil immersion lens and a 40×, 1.25 N.A. oil immersion lens with an upright confocal laser scanning microscope (SP8-UV; Leica Microsystems). Acquisition of the data was performed using LAS Leica Application Imaging software. Pictures were analyzed using ImageJ software (v1.50i; NIH).

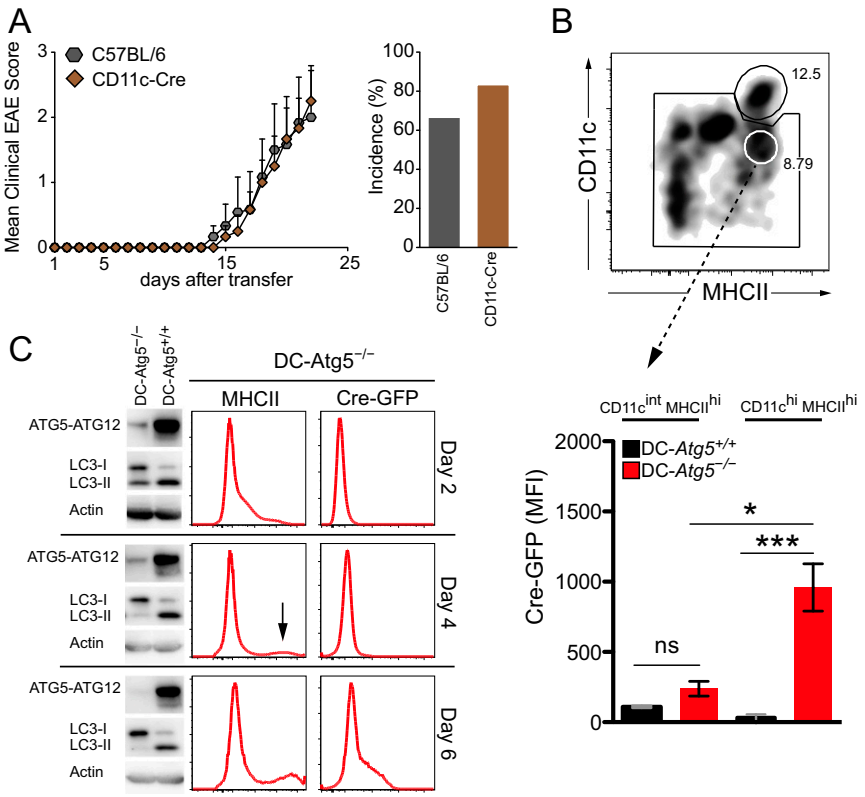

Fig. S1. (*A*) EAE was induced via adoptively transferring 2D2/TCR^{MOG}-derived encephalitogenic CD4^{+} T cells into C57BL/6 wild-type (gray hexagons) or CD11c-Cre mice (brown diamonds). Each data point represents the mean of six animals. One representative of two independent experiments is shown (*Left*). Quantification of disease incidence is shown (*Right*). (*B*) Analysis of Cre-GFP signal in CNS CD11c^{hi}MHCII^{hi} and CD11c^{int}MHCII^{hi} subpopulations in naïve DC-*Atg5*$^{+/+}$ and DC-*Atg5*$^{-/-}$ mice. (*C*) Kinetics of the ATG5–ATG12 complex, LC3-I, and LC3-II protein levels (*Left*) and analysis of MHC class II surface expression and Cre-GFP levels (*Right*) in isolated bone marrow cells cultured with GM-CSF (2.5 ng/mL). Statistical analysis: Mean ± SEM is depicted. Unpaired two-tailed Student *t* test was applied. ns, not significant: $P > 0.05$; *$P < 0.05$, ***$P < 0.001$.

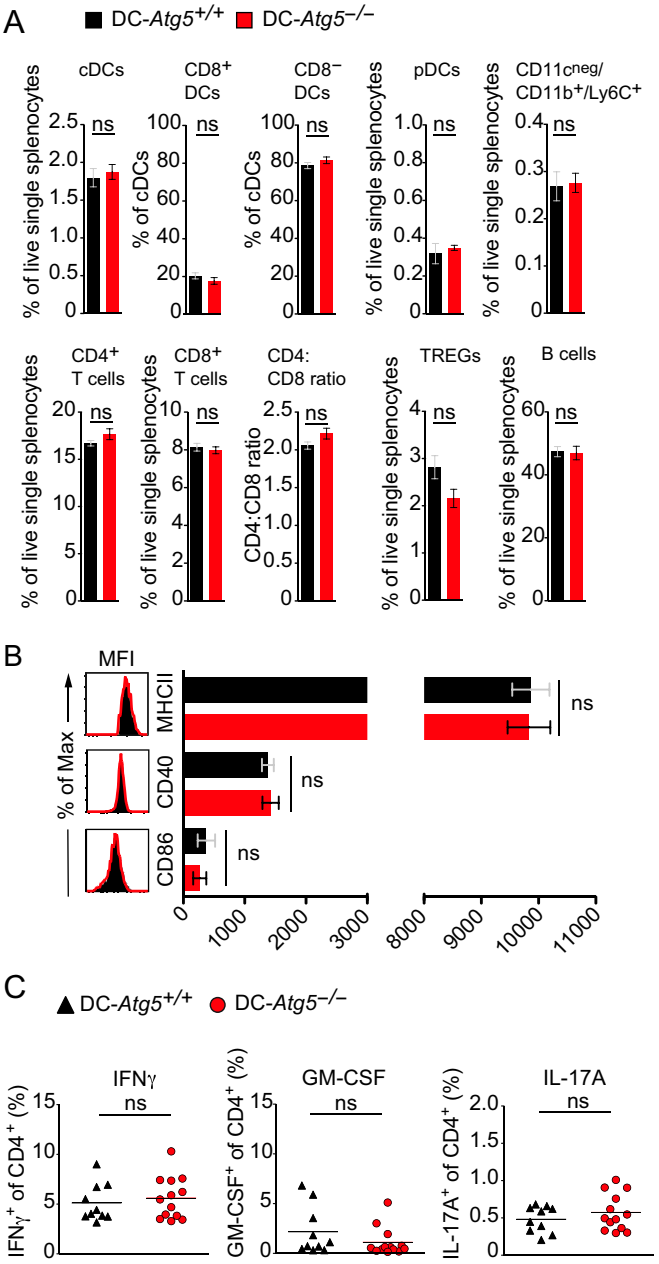

Fig. S2. Steady-state immune compartments in spleens of DC-*Atg5*$^{+/+}$ and DC-*Atg5*$^{-/-}$ mice. (*A*) Flow cytometric analysis of the splenic myeloid immune cell compartments in DC-*Atg5*$^{+/+}$ and DC-*Atg5*$^{-/-}$ mice revealed no difference in frequencies of classical DCs (cDCs; all MHCII^{+}CD11c^{+} including CD8^{-} and CD8^{+} DCs), CD8^{-} DCs, CD8^{+} DCs, plasmacytoid DCs, or monocytes (CD11c^{neg}CD11b^{+}Ly6C^{+}). Analysis of lymphocyte subsets showed no difference between the two genotypes in frequencies of CD4^{+} T cells or CD8^{+} T cells or the CD4:CD8 T cell ratio. Frequencies of Foxp3^{+} T regulatory cells (Tregs) and B cells were similar (DC-*Atg5*$^{+/+}$, $n = 7$; DC-*Atg5*$^{-/-}$, $n = 9$). (*B*) Representative histograms and quantification of median fluorescence intensity (MFI) for MHC class II (*Upper*), CD40 (*Middle*), and CD86 (*Lower*) on CD11c^{hi}MHCII^{hi} DCs. (*C*) Capability of splenic CD4^{+} T cells to produce the proinflammatory cytokines IFNγ (*Left*), GM-CSF (*Middle*), and IL-17A (*Right*) upon stimulation with PMA/ionomycin was analyzed via intracellular cytokine staining. Each dot represents an individual animal. Statistical analysis: Mean ± SEM is depicted. Unpaired two-tailed Student *t* test was applied. ns, not significant: $P > 0.05$.

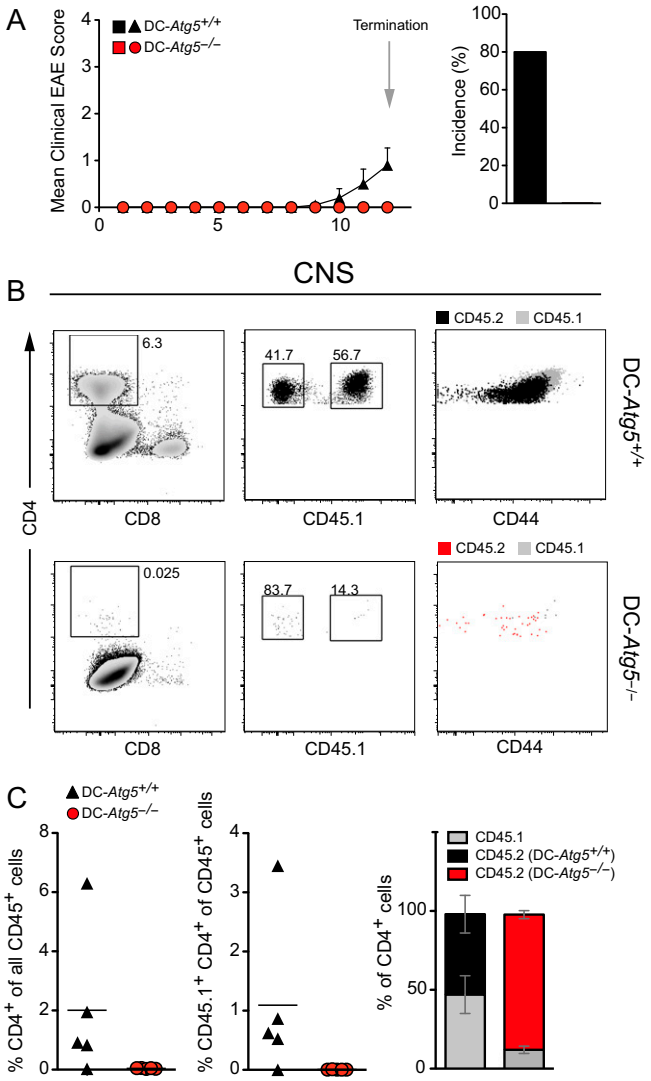

Fig. S3. Myelin-specific CD4$^+$ T cells accumulate in the CNS early upon adoptive EAE induction. (*A*) EAE was induced via adoptively transferring CD45.1 2D2/TCR^{MOG}-derived encephalitogenic CD4$^+$ T cells into DC-*Atg5*$^{+/+}$ or DC-*Atg5*$^{-/-}$ mice (both CD45.2). Each data point represents the mean of five animals. Quantification of disease incidence is shown (*Right*). (*B*) Representative density plot for CD45.1$^+$ and CD45.2$^+$ CD4$^+$ T cells in the CNS of DC-*Atg5*$^{-/-}$ or DC-*Atg5*$^{+/+}$ mice on day 12 post transfer (p.t.) (*Left* and *Middle*). CD44 expression on CD45.1$^+$ and CD45.2$^+$ CD4$^+$ T cells (*Right*). (*C*) Quantification of all CD4$^+$ T cells invading the CNS on day 12 p.t. (*Left*) and the relative fraction of CD45.1$^+$ transferred myelin-specific CD4$^+$ T cells (*Middle* and *Right*). Statistical analysis: Mean ± SEM is depicted.

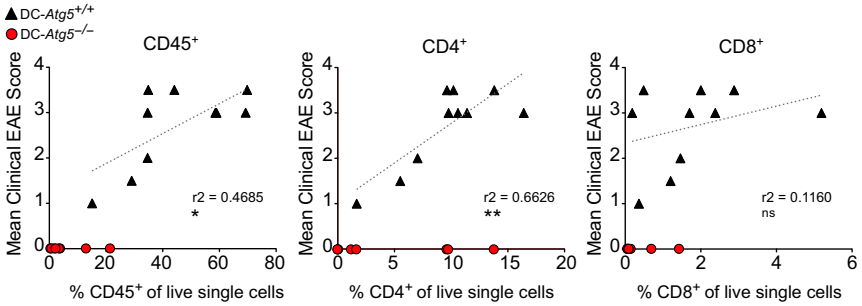

Fig. S4. Correlation analyses of the percentage CD45$^+$ cells (*Left*), percentage CD4$^+$ T cells (*Middle*), or percentage CD8$^+$ T cells (*Right*) within live single cells with EAE disease severity 21 d (±1) after adoptive transfer EAE induction. Pooled data of two independent experiments are shown. Statistical analysis: Two-tailed Pearson correlation test was applied. ns, not significant: $P > 0.05$; *$P < 0.05$, **$P < 0.01$.