SUPPLEMENTAL MATERIALS

EXTENDED MATERIALS AND METHODS

Mice

All mice used in this study were on C57BL/6J genetic background. Mice were either obtained from The Jackson Laboratories (Bar Harbor, Maine) or bred in-house. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University. CSF1R-iKO mice were generated by breeding UBC-CreERT2 (The Jackson Laboratories, Stock #007001) with *Csf1r*^{flox} mice (The Jackson Laboratories, Stock #021212). Genotyping was performed as recommended by The Jackson Laboratories. CD45.1 mice (B6.SJL-Ptprca Pepcb/BoyJ, Stock #002014) were obtained from The Jackson Laboratories.

Induction and Scoring of EAE

EAE was induced by immunization with 1:1 emulsion of PBS and complete Freund's adjuvant (CFA) containing 5 mg/mL heat killed *M. tuberculosis* (BD Biosciences) and 1 mg/mL MOG_{35-55} peptide (Genscript). Mice were immunized on both flanks by subcutaneous injection of the emulsion for a total of 200 µL. Pertussis toxin was intraperitoneally (i.p.) injected on days 0 and 2 post immunization (p.i.) at 200 ng per dose. Mice were scored according to the following scale: 0 - No clinical symptoms. 0.5 - Partial paralysis of the tail or waddling gait. 1.0 - Full paralysis of the tail. 1.5 - Full paralysis of the tail and waddling gait. 2.0 - Partial paralysis in one leg. 2.5 - Partial paralysis in both legs or one leg paralyzed. 3.0 - Both legs paralyzed. 3.5 - Ascending paralysis. 4.0 - Paralysis above the hips. 4.5 – Moribund; mouse being unable to right itself for 30 seconds. 5.0 - Death.

In vivo Treatment

BLZ945 (Selleck Chemicals and MedChemExpress) was prepared from powder in 20% captisol at 12 mg/mL. Mice were treated with 4-6 mg/day of BLZ945 by oral gavage. In initial experiments, we used BLZ945 prepared in 20% captisol, and 20% captisol as control, which were a generous gift from Novartis International AG. Recombinant CSF-1 (4 µg/dose; R&D Systems) was given to EAE mice on days 4, 8, 12, 16 p.i. by i.p. injection.

All MAb treatments were also given by i.p. injection. Prophylactic treatments with anti-CSF-1 (200 µg/dose; clone: 5A1; Bio X Cell) started on day 0 p.i. and were given every other day until disease onset, when dosing was changed to every day. In therapeutic treatments, MAb was given every day, starting on days indicated in figures, for the duration of acute phase of the disease (typically days 11-25 p.i.), then switched to every other day for the rest of the experiments. Equal amounts of control IgG1 (Clone: HPRN; Bio X Cell) were used to treat control mice. Mice were prophylactically treated with anti-IL-34 MAb (100 µg/dose; Clone: 780310; Novus Biologicals) every other day for the duration of the experiment. For mice treated therapeutically with anti-IL-34 MAb, mice were treated after onset of disease with 55 µg per day. Anti-CSF-1R MAb (400 µg/dose; Clone: AFS98; Bio X Cell) was given every other day. In experiments with anti-IL-34 and anti-CSF-1R MAbs, equal amounts of control IgG2A (Clone: 2A3; Bio X Cell) were given to control mice. Tamoxifen (Sigma) was dissolved in corn oil (Sigma) to 20 mg/mL by heating in a 37ºC water bath and vortexing. For tamoxifen treatment by i.p. injection, 2 mg of tamoxifen was injected daily for a total of 5 injections. Mice were then rested for 14-21 days before further manipulation. For tamoxifen treatment by oral gavage, mice were treated for 5 days with 5 mg of tamoxifen per day, then rested for 7 days before further manipulation.

Cell Isolation and Flow Cytometry

Mice were anesthetized and blood was harvested from abdominal aorta. For isolation of CNS mononuclear cells, blood was removed by perfusion with 60 mL PBS. Spinal cord was flushed out of the spinal column with PBS. Brains and spinal cords were pooled and cut manually into small pieces in 700 µL Liberase TL dissolved in RPMI at 0.7 mg/mL (Roche) then incubated at 37ºC for 30 min before reaction was quenched using complete media containing FBS. Tissue was homogenized by pushing through a 100 µm sterile filter with syringe plunger. Homogenate was centrifuged at 1500 RPM (300 x g) for 5 min and resuspended in 25 mL of 70% 1x Percoll-PBS (90% Percoll, 10% 10x PBS). 25 mL of 30% Percoll-PBS was gently overlayed onto the 70% layer and was centrifuged at 2000 RPM without brake at room temperature for 30 min. Cells that pooled at the interface of 30/70% layers and the majority of the 30% layer were then collected, diluted with PBS or media and centrifuged at 1500 RPM (300 x g) for 5 min. Spleen and dLNs were homogenized through a 100 µm sterile filter before RBC lysis with ammonium chloride solution (Biolegend).

Isolated cells were stimulated with PMA (50 ng/mL; Sigma Aldrich), ionomycin (500 ng/mL; Sigma Aldrich), and 1 µL/mL Golgiplug (BD Biosciences) for 4 h at 37^{o}C or stained without stimulation. After stimulation, cells were washed with PBS containing 3% FBS (v/v). Cell surface antigens were stained with Abs in 100 µL of PBS/3% FBS for 20-30 min at 4^{o}C. Cells were then washed and fixed with 100 µL Fix and Perm Medium A (Thermo Fisher) for 20 min at room temperature and washed again. Cells were permeabilized with Fix and Perm Medium B (Thermo Fisher) and stained with Abs against intracellular antigens in 100 µL Fix and Perm Medium B and 100 µL PBS/3%FBS for 1 h or overnight. Cells were then washed twice, resuspended in 500 µL PBS and analyzed on a BD FACSAria Fusion flow cytometer (BD Biosciences).

Antibody Titer Measurement

Serum was collected from peripheral blood of animals treated with Rat IgG_{2A} (Clone: 2A3; Bio X Cell), anti-CSF1 MAb (Clone: 5A1; Bio X Cell), anti-CSF-1R MAb (Clone: AFS98; Bio X Cell), anti-IL-34 MAb (Clone: 780310, Novus Biologicals). 96-well ELISA plates were coated with the same MAb that was used to treat animals overnight at room temperature and blocked with 1% BSA for 2 h. Plates were washed and incubated with serum for 1 h and washed. Anti-Mouse IgG-HRP conjugate secondary Ab (Jackson Immunolabs) was used to detect the presence of anti-Rat IgG response by measuring absorbance at 450 nm and subtracting absorbance at 540 nm.

Library Preparation and RNA-seq Analyses

Next-generation sequencing libraries were prepared using the Illumina TruSeq Stranded Total RNA library preparation kit, with high quality RNA (RIN > 8.7) and 200 ng of input RNA. Libraries were assessed for quality using the PerkinElmer Labchip GX and qPCR using the Kapa Library Quantification Kit and the Life Technologies Viia7 Real-time PCR instrument. Libraries were diluted to 2 nM and sequenced in a paired-end (2 x 100bp), dual-indexed format on the Illumina HiSeq2500 using the High Output v4 chemistry.

RNA-seq reads were demultiplexed into sample-specific fastq files and aligned to the mm10 reference genome using the DRAGEN genome pipeline to produce BAM files. Generated BAM files were read into R statistical

computing environment and gene counts were obtained using the Rsubread package, producing a feature/gene counts matrix. Differential expression analysis was performed using the R/Bioconductor package DESeq2 [1], which uses a negative binomial model. Analysis was performed using standard thresholds and parameters while filtering genes with low mean normalized counts. Further downstream analysis was performed using the Ingenuity Pathway Analysis (IPA) and GSEA software using the normalized read count table. Additionally, differentially expressed genes ($p<0.01$) were entered into the DAVID utility for functional annotation and analyzed for gene ontology terms for biological processes and for KEGG pathway terms [2, 3].

Immunohistochemistry and Immunofluorescence Microscopy

Animals were perfused with 50 mL PBS followed by 40 mL cold paraformaldehyde (PFA) solution. The spinal column was then removed and placed in PFA overnight. The next day, tissue was transferred to 30% sucrose in PBS and allowed to incubate overnight, or until tissue had sunk to the bottom of the tube. Spinal cord was then dissected from the spinal column, cut into 4 pieces of approximately equal length and embedded in OCT. The entire spinal cord was sectioned into 10 µm-thick coronal sections, skipping 100 µm between each section. Prior to staining, sections were immersed in PBS for 10 min to remove residual OCT. For Sudan black staining, sections were stained by immersion for 30 min in a 10 mg/mL solution of Sudan black prepared in 70% ethanol. Following staining, slides were decolorized by immersion in a 0.1% Triton-X100 in PBS solution for 30 min. For immunofluorescence experiments, slides were blocked using 10% normal goat serum with 0.1% sodium azide (Thermo Fisher) for 30 min. Primary Abs for Iba1 (1:500 dilution, SySy, Cat# 234 006), and CD68 (1:100 dilution, Thermo Fisher, Clone: FA-11) were diluted in 10% normal goat serum with 0.3% Triton-X100. Slides were stained with primary Abs overnight at 4^{o}C, then washed 3 times with PBS for 5 min. Secondary Abs (Thermo Fisher) were diluted 1:1000 and slides were stained for 1 h. Slides were then washed 3 times with PBS, mounted with ProLong™ Diamond Antifade Mountant with DAPI (Thermo Fisher) and imaged using a Nikon AR1 confocal microscope at the Thomas Jefferson University Imaging core facility.

Analysis of Immunofluorescence Microscopy

Demyelination in Sudan black-stained spinal cord sections was determined by dividing total demyelinated area of white matter by total white matter area, using NIS elements software (Nikon). Immunofluorescence images were analyzed using Slidebook 6 software (Intelligent Imaging Innovations). Quantification of cell density was performed by masking cell nuclei by fluorescent thresholding. Touching nuclei were separated by applying the watershed algorithm at 25% aggressiveness and then again at 50% after removal of small objects. Errors in object identification or over-segmentation caused by the watershed algorithm were then corrected manually. Features of spinal cord sections were manually masked (e.g. lesions, grey matter, etc.) and cells not belonging to that feature were discarded by only keeping cells that overlapped with the feature mask. Object IDs were then assigned to individual cells and mean pixel intensity per object was determined for each channel. A threshold intensity value was then assigned for each channel and used to determine positivity for that marker. The number of positive cells was then divided by the area of that feature to determine cell density.

Western Blot

Cells were re-suspended in a RIPA lysis buffer supplemented with a protease inhibitor cocktail (Sigma-Aldrich). Protein concentrations in cell lysates were measured with bicinchoninic acid (BCA) assay (Pierce), and 30 μg of proteins diluted with Laemmli buffer were loaded onto 8%–14% polyacrylamide gels. Anti-CSF-1R (Abcam) and mouse anti-β actin (Sigma) were used as primary antibodies.

Bone Marrow-Derived DC Culture and T cell Co-culture Assay

Bone marrow (BM) was isolated from tibia and femurs of mice, and BM cells were cultured at 1 x 10^6 cells per mL in a total volume of 10 mL in petri dishes with GM-CSF (20 ng/mL) + IL-4 (20 ng/mL) for 4 days. On the 4^{th} day, 5 mL of media was removed from the plates, cells were pelleted by centrifugation and resuspended in 5 mL fresh media containing GM-CSF and IL-4. The cell suspension was added back to the original petri dishes and cultured for an additional 3 days. To induce a mature DC phenotype, cells were washed, replated in fresh media containing LPS (300 ng/mL) and cultured for 72 h. For cultures involving monocytes, $CD11b^+$ cells were isolated

from BM cell suspension of CD45.1$^+$ mice by MACS positive selection (Miltenyi Biotec), after which Ly6C^{Hi}Ly6G$^-$ monocytes were sorted on a FACSAria Fusion instrument (BD Biosciences). Isolated monocytes were then added to CD45.2$^+$ total BM cell cultures.

For proliferation assays, 4 x 10^4 DCs were co-cultured with 1.6 x 10^5 2D2 CD4$^+$ T cells in 96-well U-bottom tissue culture plates. Cells were stimulated with MOG_{35-55} peptide (25 μg/mL) for 72 h. At approximately 60 h after starting the culture, 1 μCi of [^{3}H]Thymidine (Perkin-Elmer) was added to each well in a volume of 50 μL. Cells were harvested 24 h later and counts per minute (C.P.M.) measured in MicroBeta2 beta counter (Perkin-Elmer).

Bone Marrow Chimeras

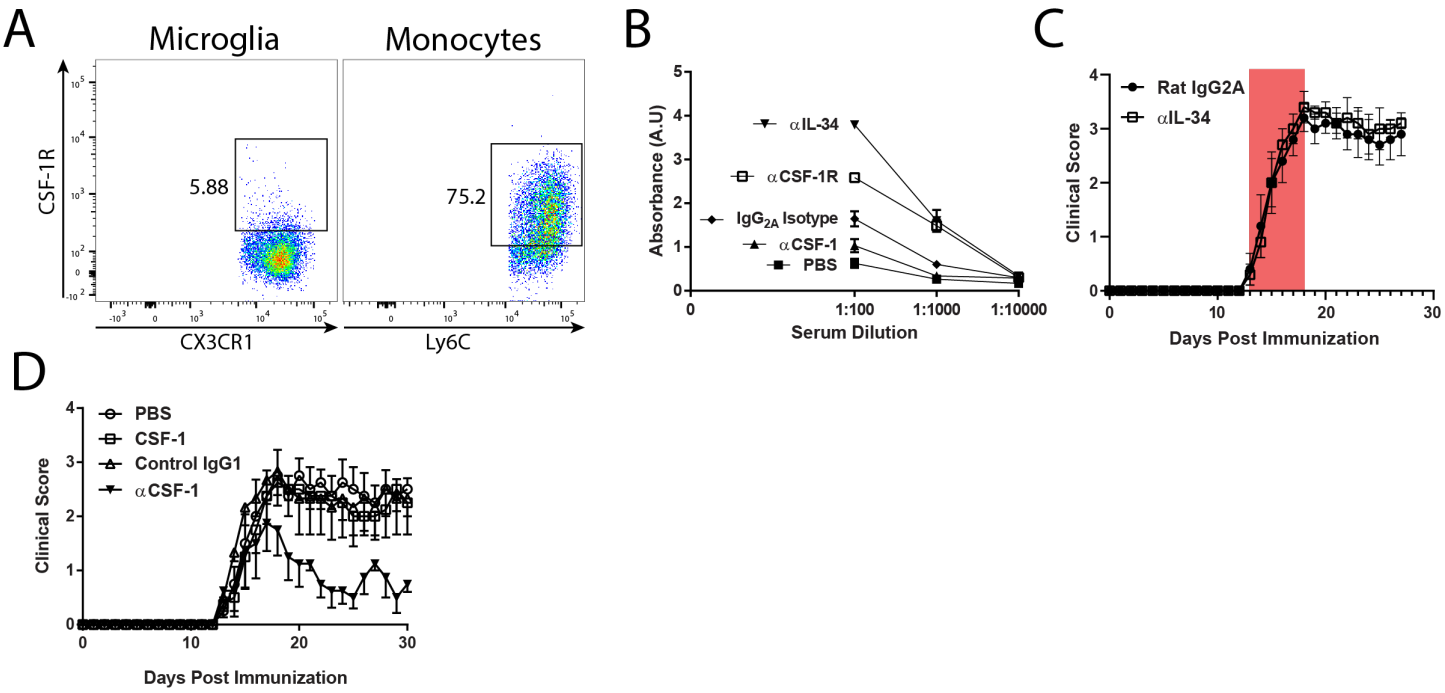
Treosulfan (a generous gift from Medac Germany) was resuspended in sterile water at a concentration of 50 mg/mL and recipient mice were injected i.p. with 2000 mg/kg treosulfan once per day for 3 consecutive days. Mice were then rested for 72-96 h before i.v. transfer of 8-10 x 10^6 BM cells. For BM chimera experiments where WT or CSF1R-iKO mice were reconstituted with either WT or CSF1R-iKO BM, WT CD45.1$^+$ (from B6.SJL-Ptprca Pepcb/BoyJ mice) and CSF1R-iKO (CD45.2$^+$), BM was transferred into either WT (CD45.2$^+$) or CSF1R-iKO (also CD45.2$^+$) recipients. Recipient mice were allowed to reconstitute for 8 weeks before treatment with tamoxifen by oral gavage (5 mg per day for 5 consecutive days), to induce knockout of *Csf1r*. Mice were then allowed to rest for 7 days before immunization with MOG_{35-55} to induce EAE. For mixed BM chimeras, recipient WT CD45.1 mice were reconstituted with a 1:1 mixture of CD45.1:CSF1R-iKO BM (8 x 10^6 cells were transferred) or WT CD45.1:CD45.2 BM and rested for 8 weeks before tamoxifen treatment and EAE induction as described above.

References

1. Love, M.I., W. Huber, and S. Anders, *Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2.* Genome Biol, 2014. **15**(12): p. 550. PMC4302049 PMID: 25516281.
2. Huang da, W., B.T. Sherman, and R.A. Lempicki, *Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.* Nat Protoc, 2009. **4**(1): p. 44-57. PMID: 19131956.
3. Huang da, W., B.T. Sherman, and R.A. Lempicki, *Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists.* Nucleic Acids Res, 2009. **37**(1): p. 1-13. PMC2615629 PMID: 19033363.

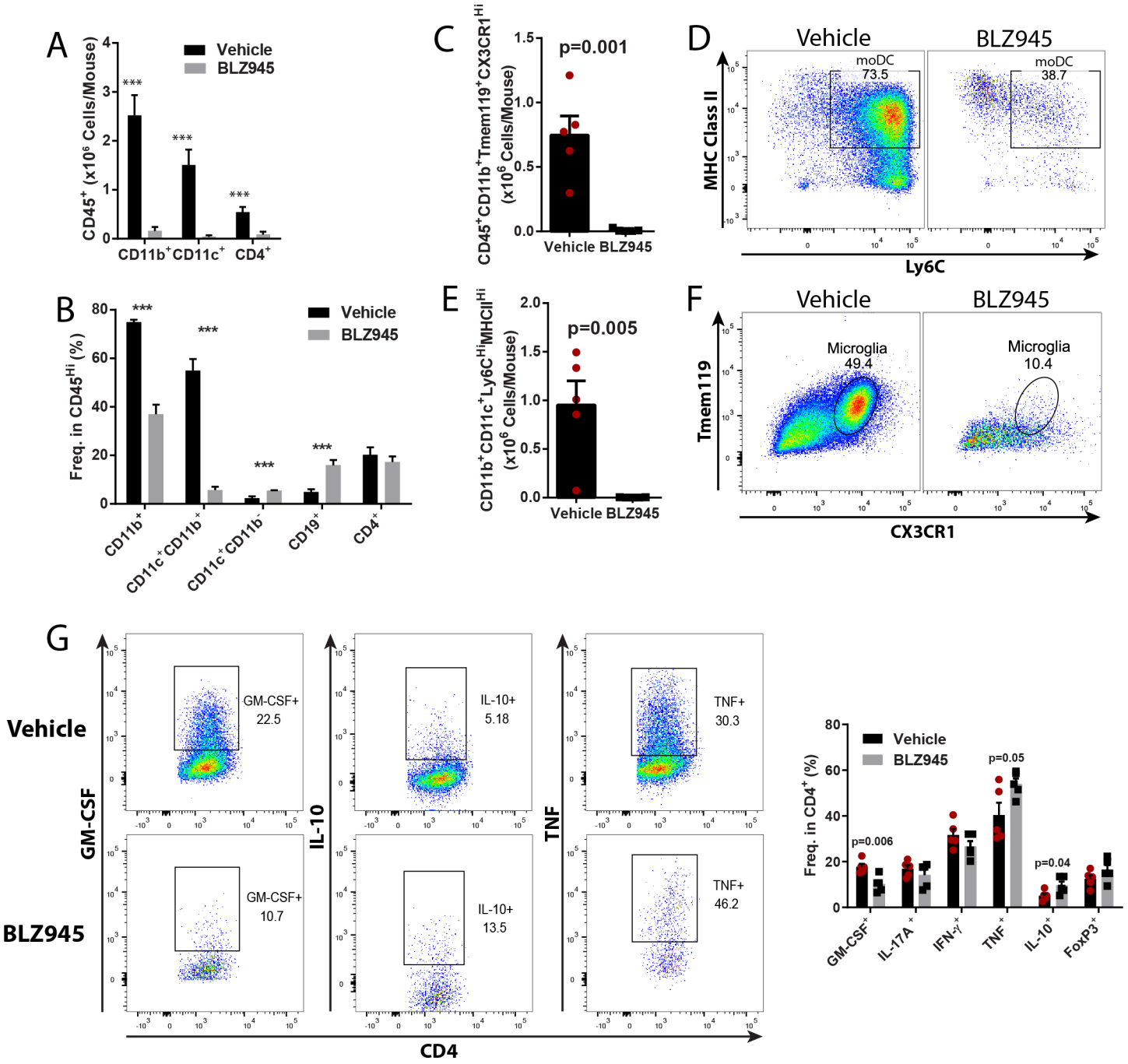
SUPPLEMENTAL FIGURES

Supplemental Figure 1



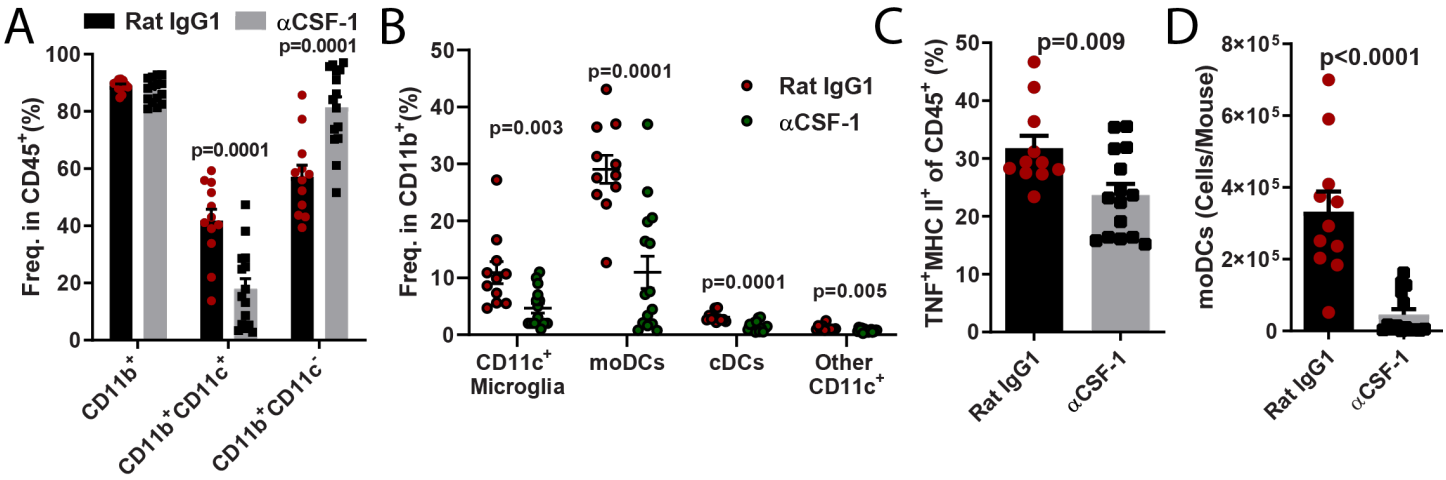
Supplemental Figure 1: A) Flow cytometry plots depicting staining of $CD45^{+}CD11b^{+}CX3CR1^{Hi}$ microglia and $CD45^{Hi}CD11b^{+}Ly6G^{-}Ly6C^{Hi}$ monocytes for CSF-1R with the AFS98 MAb in EAE mice. **B)** Serum Ab titers of mice with EAE treated with MAbs against IL-34 (n=3), CSF-1R (n=3), IgG2a isotype (n=4), CSF-1 (n=3), and control animals treated with PBS (n=6). Error bars are standard error from the mean. **C)** C57BL6/J mice were immunized with MOG_{35-55} and treated with αIL-34 MAb (55 µg per day, i.p) for the period indicated by the red box. **D)** C57BL6/J mice were immunized with MOG_{35-55} and treated with αCSF-1 MAb (200 µg, every other day), control rat IgG1 MAb (200 µg, every other day), recombinant mouse CSF-1 (4 µg per dose, given on days 4, 8 ,12 ,16 p.i.) or PBS by i.p. injection.

Supplemental Figure 2



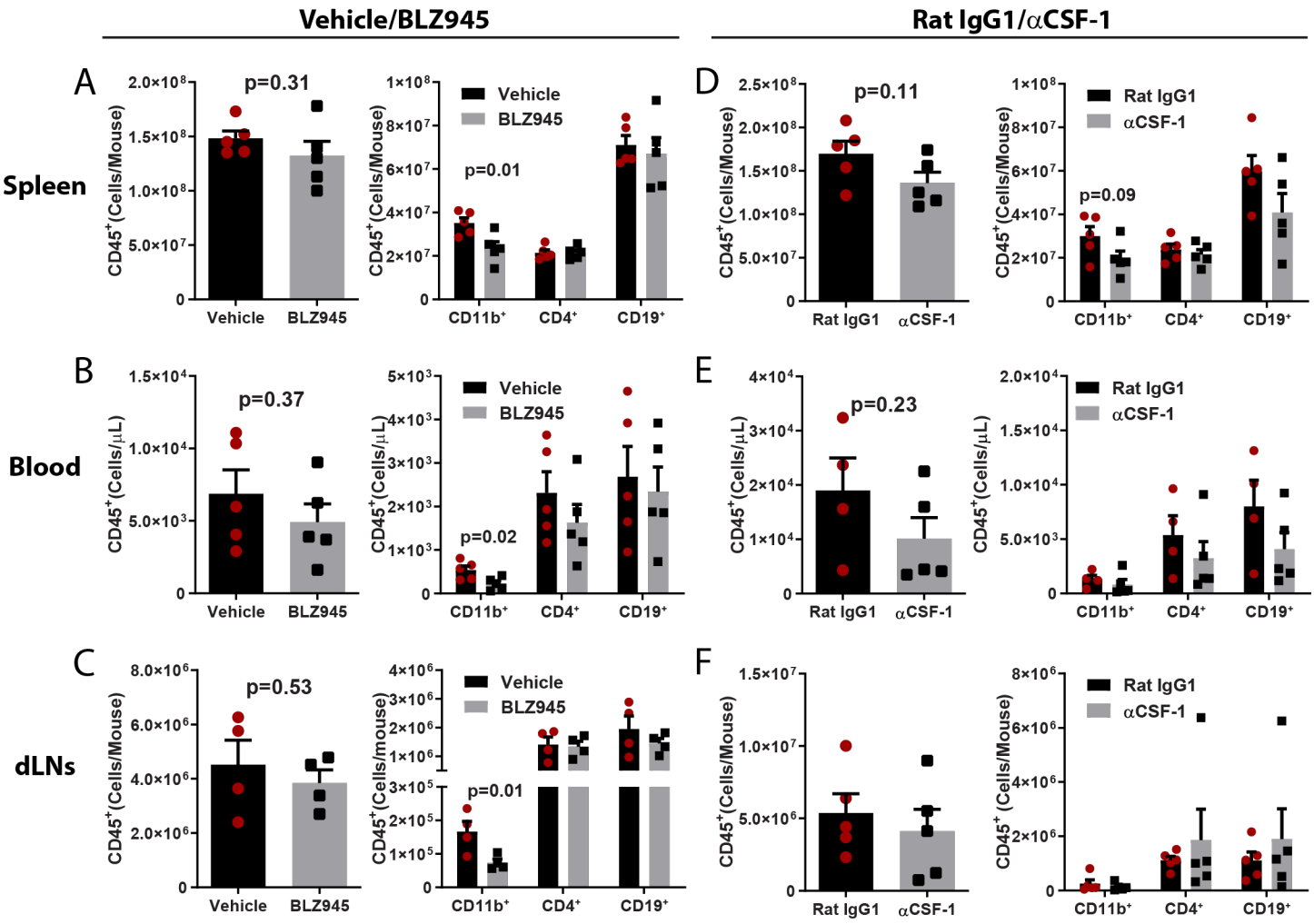
Supplemental Figure 2: Inhibition of CSF-1R signaling alters both myeloid and T cell compartments in the CNS of EAE mice. C57BL/6J mice were immunized with MOG_{35-55} for EAE induction and treated orally with BLZ945 (200 mg/kg/day) or vehicle control (20% captisol) daily, starting on the day of immunization. Mice were sacrificed on day 15 p.i., and pooled brain and spinal cords of each mouse were used for cell isolation (n=5/group). **A)** Numbers of $CD11b^+$, $CD11c^+$ and $CD4^+$ cells. **B)** Frequency of $CD11b^+$, $CD11b^+CD11c^+$, $CD11b^+CD11c^-$, $CD19^+$, and $CD4^+$ cells in $CD45^{Hi}$ cells. **C-F)** Numbers of $CD45^+CD11b^+Tmem119^+CX3CR1^{Hi}$ microglia and $CD45^{Hi}CD11b^+Ly6C^{Hi}MHCII^{Hi}$ moDCs. **G)** Quantification of GM-CSF, IL-17A, IFN-γ, TNF, IL-10 and FoxP3 expression by $CD4^+$ cells from the CNS. Significance was calculated with Student's *t* test. Error bars are S.E.M.

Supplemental Figure 3



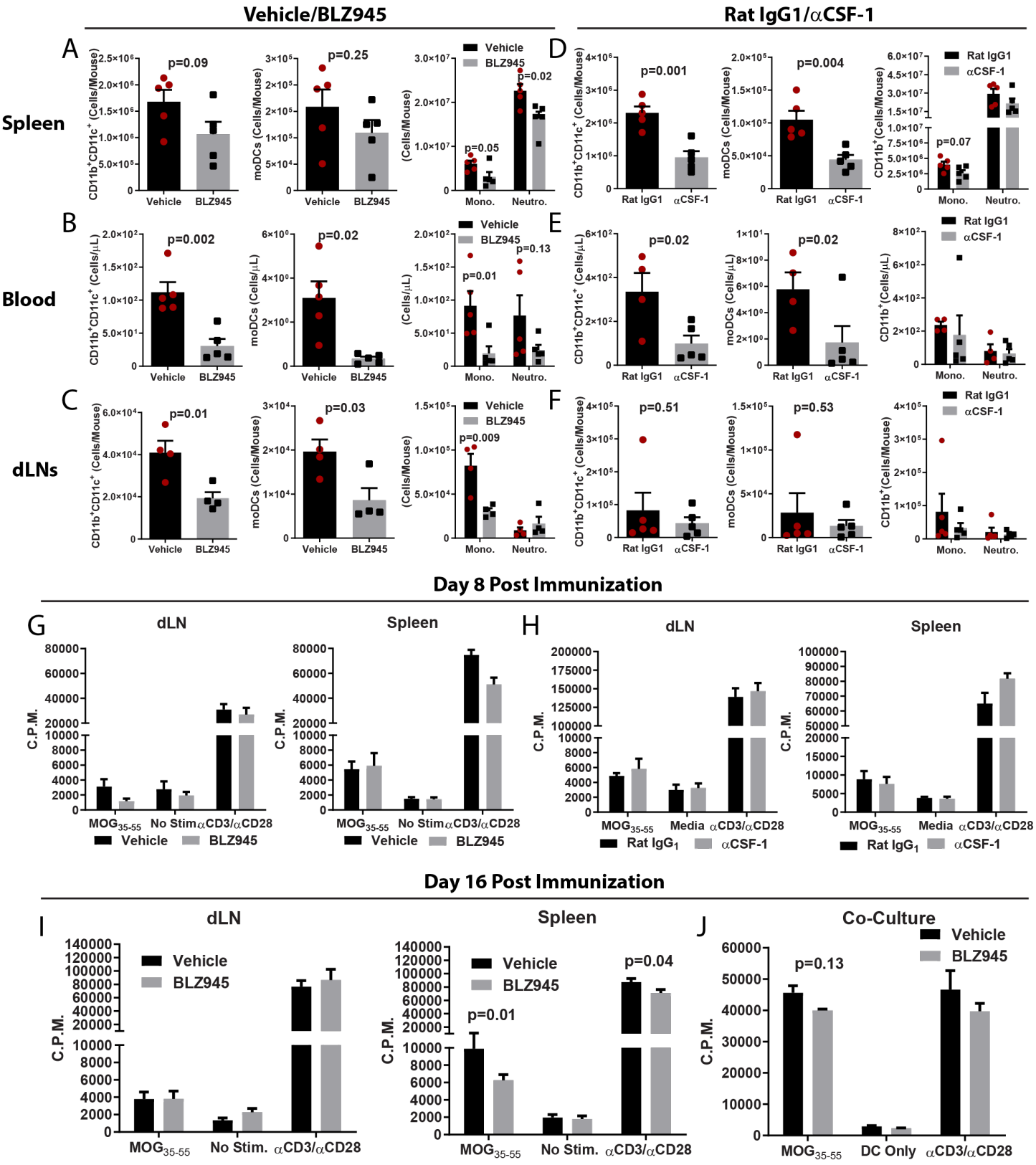
Supplemental Figure 3: Anti-CSF-1 treatment reduces numbers of DCs in the CNS of mice with EAE. A) Frequency of $CD11b^+$, $CD11b^+CD11c^+$, $CD11b^+CD11c^-$ cells among $CD45^+$ cells from the CNS of mice with EAE treated with either αCSF-1 or control MAb. **B)** Frequency of $CD11c^+$ microglia ($CD45^+CD11b^+CX3CR1^{Hi}Tmem119^+$), moDCs ($CD45^{Hi}CD11b^+CD11c^+Ly6G^-Ly6C^{Hi}MHCII^{Hi}$), cDCs ($CD45^{Hi}CD11b^+CD11c^+CD26^+Ly6C^-MHCII^+$) and other $CD11c^+$ cells among $CD11b^+$ cells. **C)** Number of $CD45^{Hi}CD11b^+CD11c^+Ly6G^-Ly6C^{Hi}MHCII^{Hi}$ moDCs. **D)** Frequency of TNF^+MHCII^+ cells among $CD45^+$ cells. Significance was calculated with unpaired Student's *t* test. Error bars are S.E.M.

Supplemental Figure 4



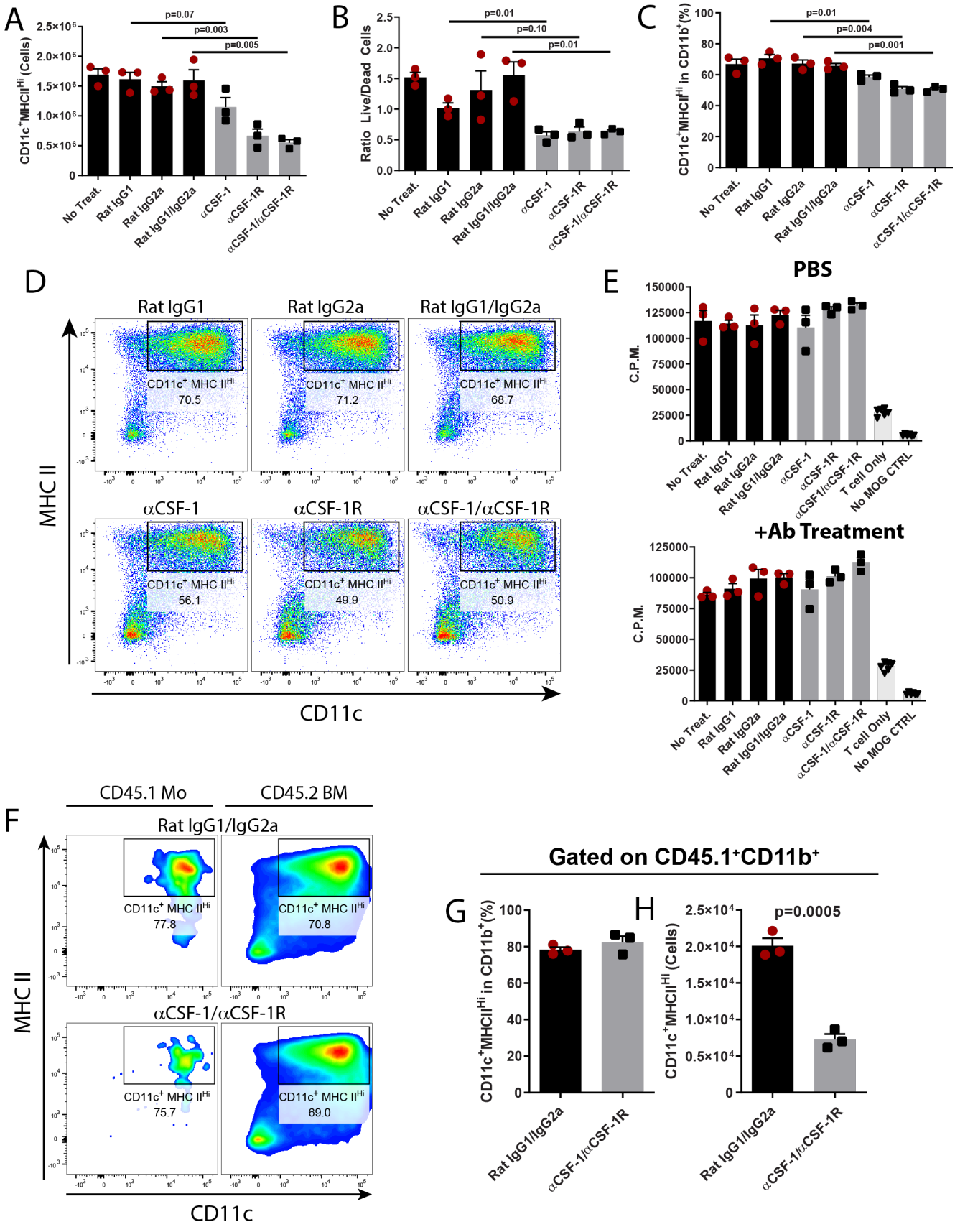
Supplementary Figure 4: Immune response in peripheral lymphoid organs of BLZ945- and αCSF-1-treated mice on day 8 p.i. A-C) BLZ945- and vehicle-treated mice (n=5 per group). Number of $CD45^+$, $CD11b^+$, $CD4^+$ and $CD19^+$ cells in **A)** spleen, **B)** blood, and **C)** dLN. **E-F)** Same quantification as in A-C), but for αCSF-1- and rat IgG1 control MAb-treated mice. Statistical significance was calculated using two-way unpaired *t* test. Error bars are standard S.E.M.

Supplemental Figure 5



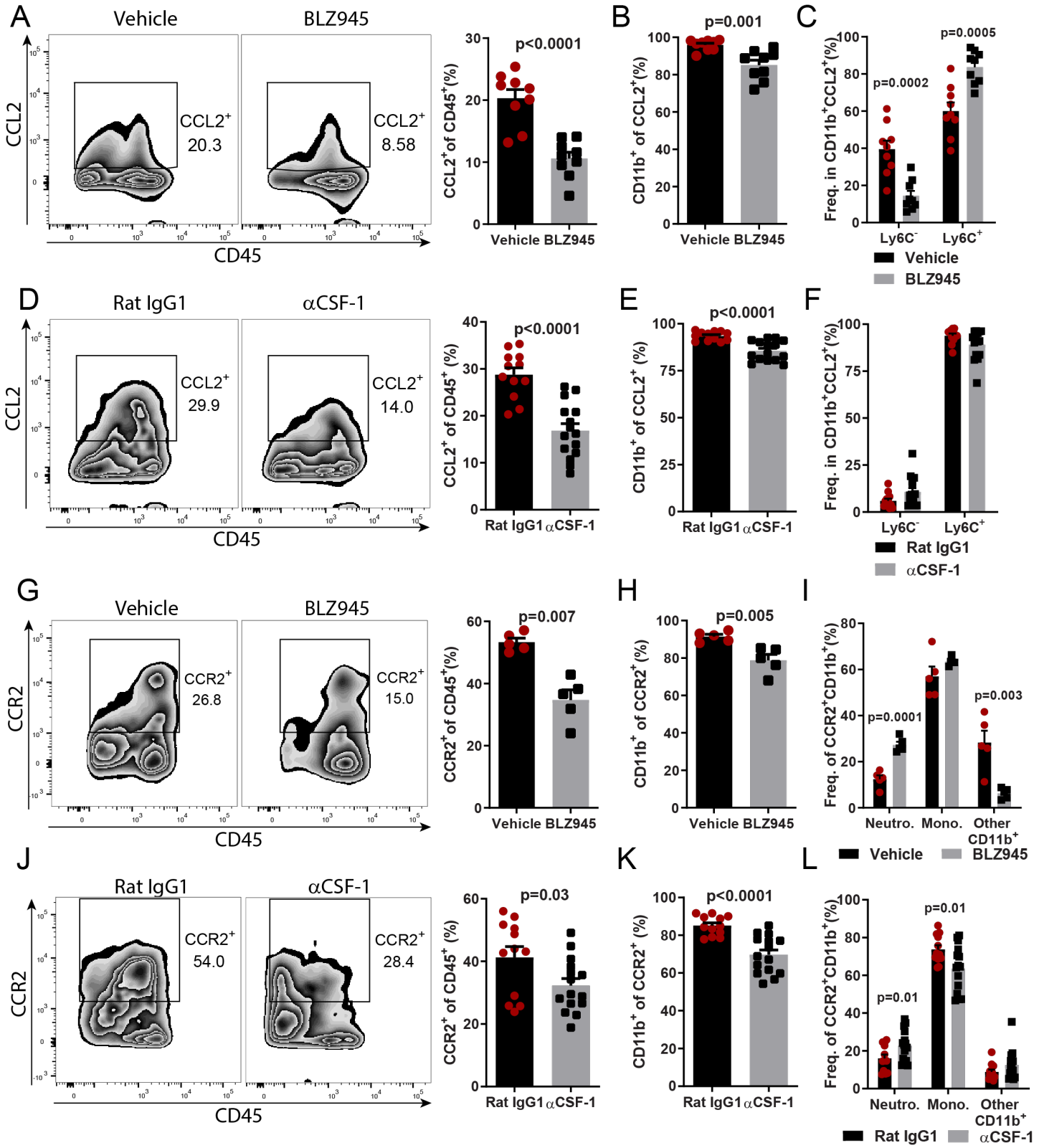
Supplemental Figure 5: CSF-1R inhibition depletes myeloid DCs and monocytes in peripheral lymphoid compartments. Characterization of immune cells in the spleen, blood and dLNs from MOG_{35-55}-immunized mice sacrificed on day 8 p.i.. **A-C)** BLZ945- and vehicle-treated mice (n=5 per group). Numbers of $CD11b^+CD11c^+$, moDCs, monocytes and neutrophils in **A)** spleen, **B)** blood, and **C)** dLN. **E-F)** Same quantification as in A-C) but for αCSF-1- and rat IgG1 control MAbs-treated mice. **G)** [^{3}H]Thymidine proliferation assay for MOG_{35-55}-stimulated splenocytes and dLNs cells from BLZ945- and vehicle-treated mice harvested on day 8 p.i. C.P.M. = counts per minute. **H)** Same analyses as in G), but for αCSF-1- and rat IgG1 control MAb-treated mice. **I)** [^{3}H]Thymidine proliferation assay for MOG_{35-55}-stimulated splenocytes and dLNs cells from BLZ945- and vehicle-treated mice harvested on day 16 p.i. **J)** $CD11c^+$ cells in splenocytes from BLZ945- and vehicle-treated mice harvested on day 8 p.i. $CD11c^+$ cells were isolated by magnetic bead sorting and mixed in a 1:10 ratio with $CD4^+$ T cells isolated from splenocytes of 2D2 mice, and stimulated with MOG_{35-55} for 72 h. Proliferation was then measured by [^{3}H]Thymidine incorporation assay. Statistical significance was calculated using two-way unpaired *t* test. Error bars are S.E.M.

Supplemental Figure 6



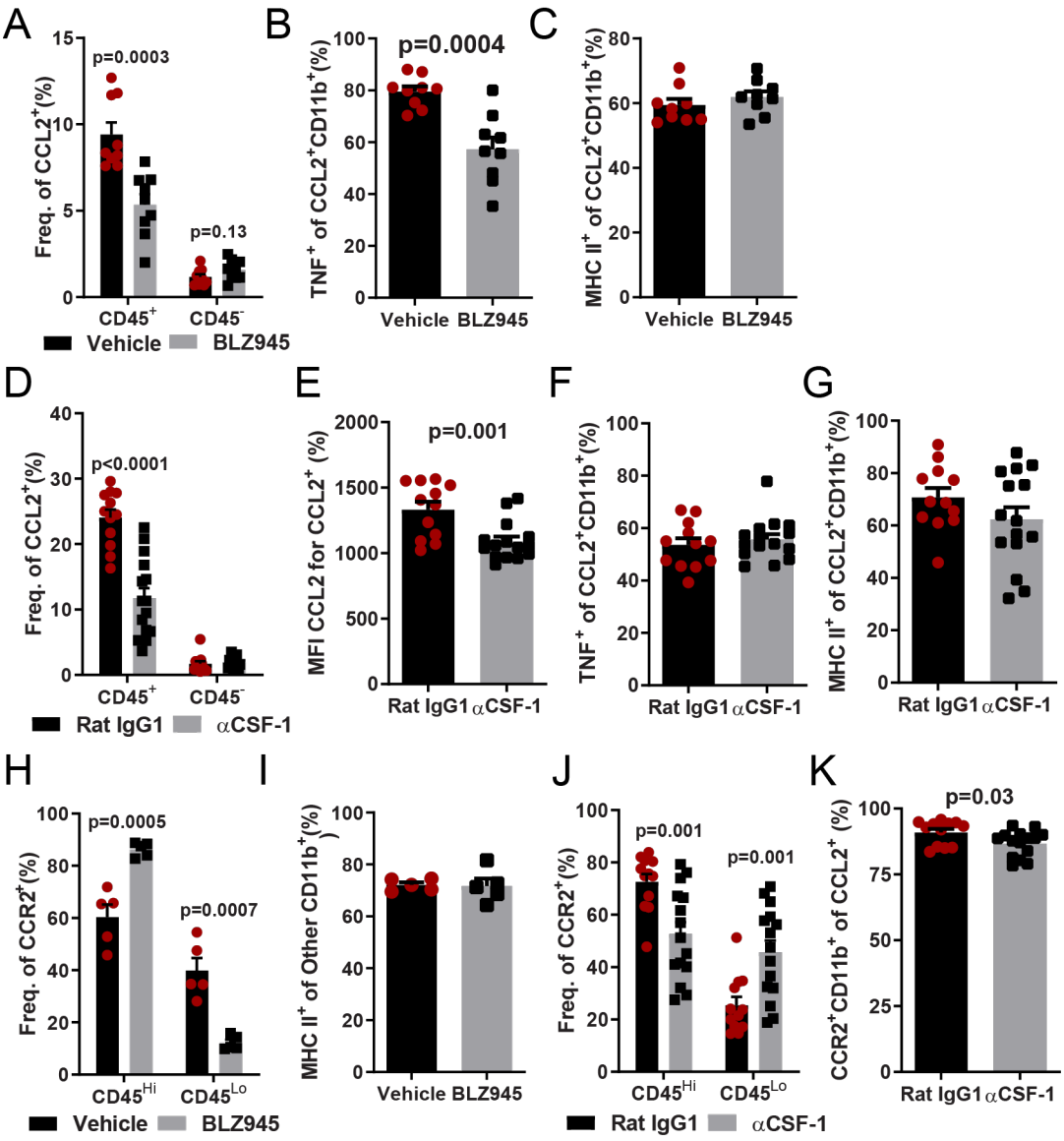
Supplemental Figure 6: CSF-1R signaling promotes survival and proliferation of moDCs, but not their APC function. BM cells were cultured in media supplemented with GM-CSF and IL-4 for 7 days in the presence of either control MAbs, or αCSF-1 and αCSF-1R MAbs. BMDCs were then matured by stimulation with LPS for 24 h in the presence of the MAbs. **A)** Number of $CD11c^{+}MHCII^{Hi}$ cells in culture after GM-CSF + IL-4 treatment for 7 days. **B)** Ratio of Live/Dead cells after 24 h LPS treatment. **C-D)** Frequency of $CD11c^{+}MHCII^{Hi}$ cells after GM-CSF + IL-4 treatment for 7 days. **E)** Co-culture of LPS-matured BMDCs with $CD4^{+}$ T cells from 2D2 mice and MOG_{35-55} peptide (25 μg/mL). Co-cultures either did, or did not contain control and neutralizing MAbs against CSF-1 and CSF-1R. Proliferation was measured using [^{3}H]Thymidine incorporation. C.P.M. = counts per minute. **F)** Monocytes were purified from the BM of CD45.1 mice and mixed with total BM of CD45.2 mice, then cultured as described above with control or αCSF-1/αCSF-1R MAbs. Flow cytometry depicting CD11c and MHC II expression in $CD45.1^{+}CD11b^{+}$ and $CD45.2^{+}CD11b^{+}$ cells is shown. **G)** Frequency, and **H)** number of $CD11c^{+}MHC\ II^{Hi}$ cells among $CD45.1^{+}CD11b^{+}$ cells. Technical replicates for 1 of 2 independent experiments with similar results are shown. Statistical significance was calculated using two-way unpaired *t* test. Error bars are S.E.M.

Supplemental Figure 7



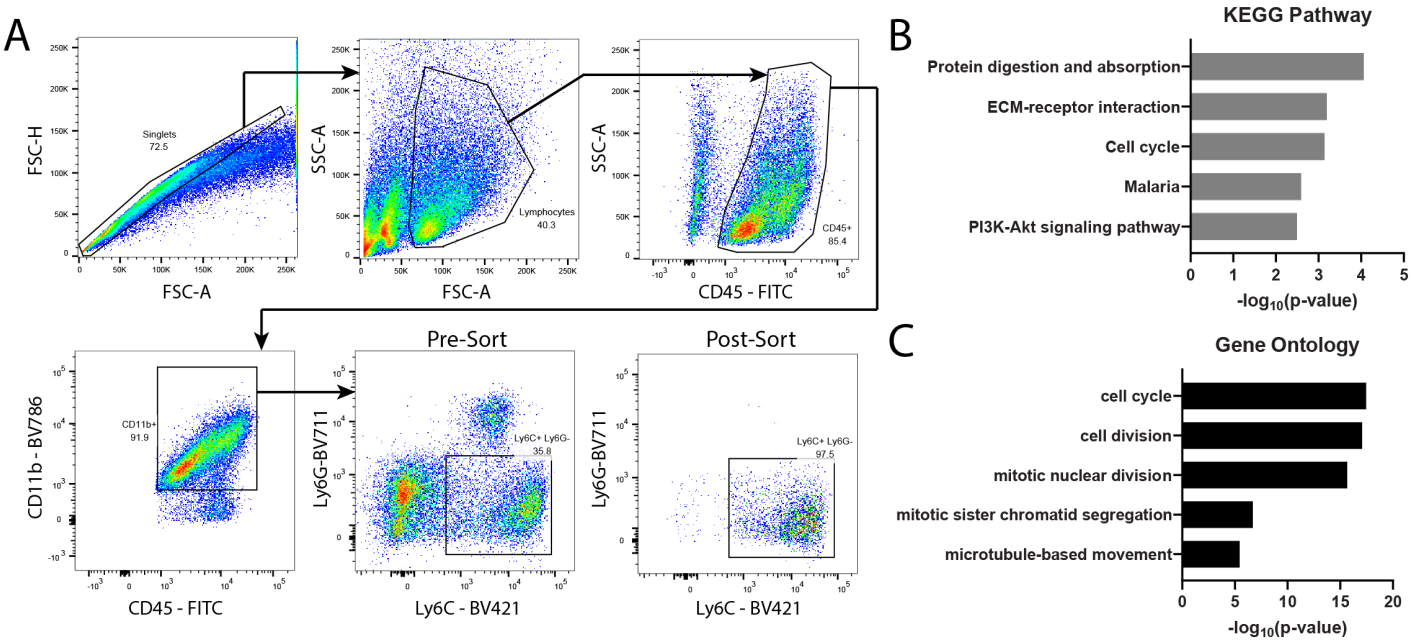
Supplemental Figure 7: Blocking CSF-1R or CSF-1 reduces numbers of $CCL2^+$ and $CCR2^+$ myeloid cells in the CNS during EAE. A) Frequency of $CCL2^+$ cells among $CD45^+$ cells in the CNS of BLZ945- and vehicle-treated mice (n=9 per group) sacrificed after 6 days of treatment, at day 20 p.i.. **B)** $CD11b^+$ cells among $CCL2^+$ cells. **C)** Frequency of $Ly6C^+$ and $Ly6C^-$ cells among $CD11b^+CCL2^+$ cells. **D)** Frequency of $CCL2^+$ cells among $CD45^+$ cells from the CNS of αCSF-1- and control MAb-treated mice (n=12-15 per group), sacrificed after 6 days of treatment, at 17 days p.i.. **E)** $CD11b^+$ cells among $CCL2^+$ cells. **F)** Frequency of $Ly6C^+$ and $Ly6C^-$ cells among $CD11b^+CCL2^+$ cells. **G)** Frequency of $CCR2^+$ cells among $CD45^+$ cells from the CNS of BLZ945- and vehicle-treated mice from A). **H)** $CD11b^+$ cells among $CCL2^+$ cells. **I)** Frequency of neutrophils ($CD45^{Hi}CD11b^+Ly6G^{Hi}Ly6C^{Int}$), monocytes/monocyte-derived cells ($CD45^{Hi}CD11b^+Ly6G^{Neg/Lo}Ly6C^+$) and other $CD11b^+$ cells among $CCR2^+CD11b^+$ cells. **J)** Frequency of $CCR2^+$ cells among $CD45^+$ cells from the CNS of αCSF-1- and control MAb-treated mice from D). **K)** $CD11b^+$ cells among $CCL2^+$ cells. **L)** Frequency of neutrophils ($CD45^{Hi}CD11b^+Ly6G^{Hi}Ly6C^{Int}$), monocytes/monocyte-derived cells ($CD45^{Hi}CD11b^+Ly6G^{Neg/Lo}Ly6C^+$) and other $CD11b^+$ cells among $CCR2^+CD11b^+$ cells. Statistical significance was calculated using two-tailed unpaired *t* test. Error bars are S.E.M.

Supplemental Figure 8



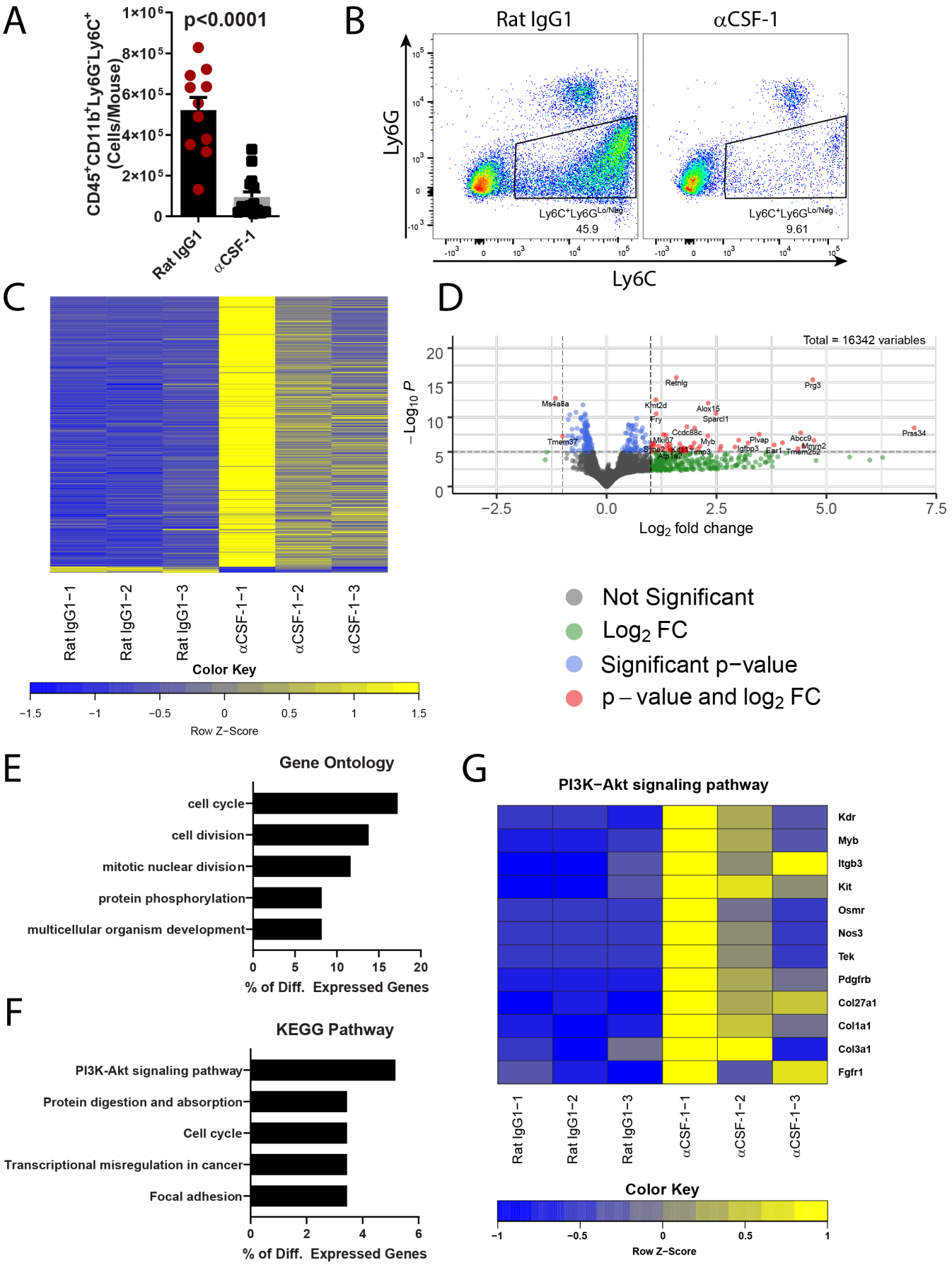
Supplemental Figure 8. CCL2⁺ and CCR2⁺ cells are primarily composed of inflammatory myeloid cells. Frequency of $CCL2^+CD45^+$ and $CCL2^+CD45^-$ cells among mononuclear cells isolated from the CNS of BLZ945- and vehicle-treated mice with EAE. **B)** Frequency of TNF^+, and **C)** $MHCII^+$ cells among $CCL2^+CD11b^+$ cells. **D)** Frequency of $CCL2^+CD45^+$ and $CCL2^+CD45^-$ cells among mononuclear cells isolated from the CNS of αCSF-1- and control MAb-treated EAE mice. **E)** MFI of $CCL2^+$ cells among $CD45^+CCL2^+$ cells. **F)** Frequency of TNF^+, and **G)** $MHCII^+$ cells among $CCL2^+CD11b^+$ cells. **H)** Frequency of $CCR2^+$ cells among $CD45^+$ cells that were either $CD45^{Hi}$ or $CD45^{Lo}$ in BLZ945- or vehicle-treated EAE mice. **I)** Percentage of $MHCII^+$ among "Other $CD11b^+$ cells" from Fig. 7B. **J)** Frequency of $CCR2^+$ cells among $CD45^+$ cells that were either $CD45^{Hi}$ or $CD45^{Lo}$ in αCSF-1- or control MAb-treated mice. **K)** Frequency of $CCR2^+CD11b^+$ cells among $CCL2^+$ cells. Statistical significance was calculated with two-tailed unpaired *t* test. Error bars are S.E.M.

Supplemental Figure 9



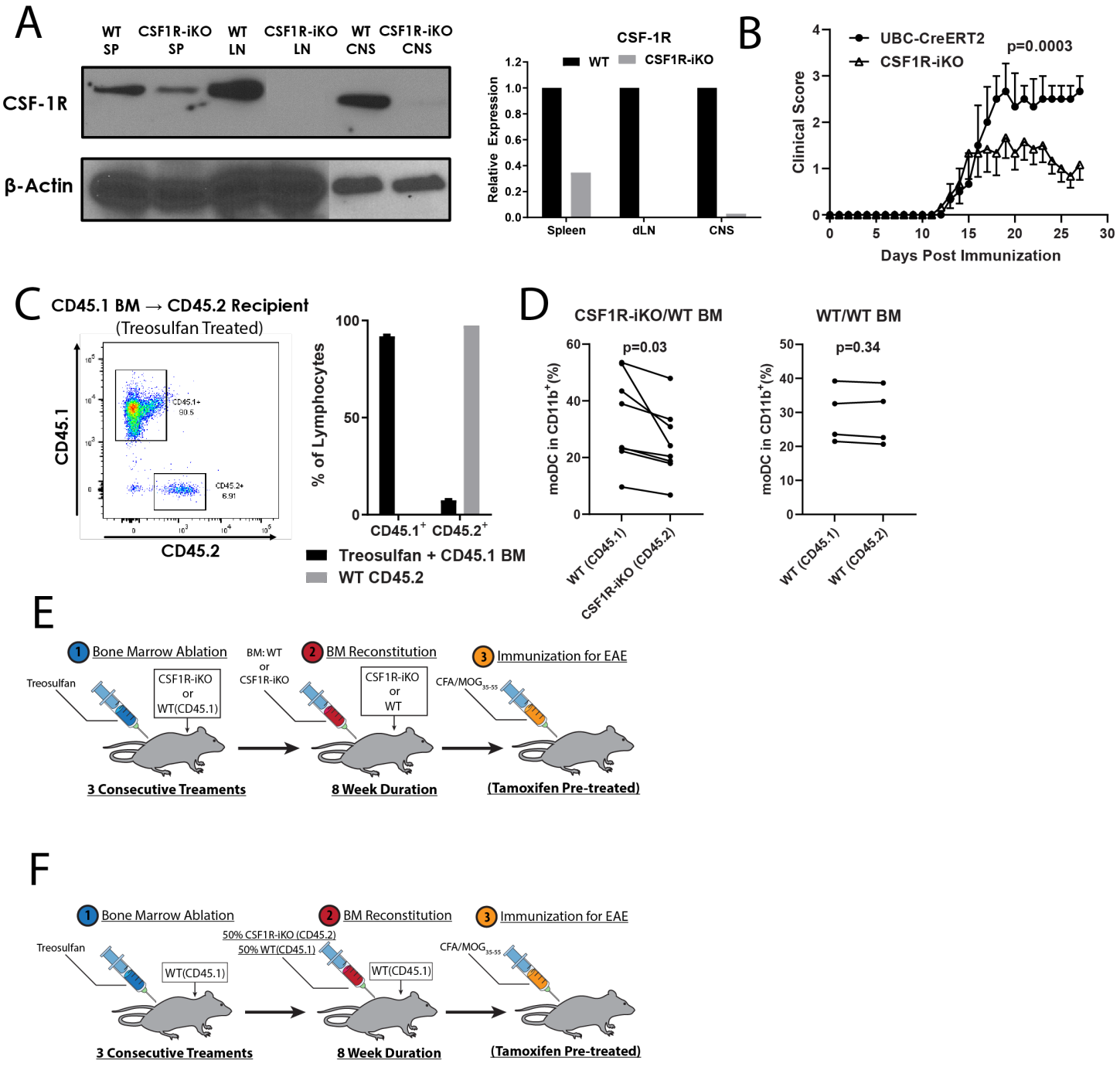
Supplemental Figure 9. Monocytes from the CNS of mice with EAE treated with αCSF-1 MAb have distinct transcriptional profile. EAE was induced in C57BL/6J mice by immunization with MOG_{35-55}. Mice were treated with 200 μg/day of αCSF-1 MAb or isotype control MAb from day 11 to 16 p.i. and sacrificed on day 17 p.i. **A)** Gating strategy for FACS sorting of $CD45^{Hi}CD11b^{+}Ly6G^{-}Ly6C^{+}$ monocytes/monocyte-derived cells. **B)** Gene ontology and **C)** KEGG pathway terms ranked by significance and generated from differentially expressed genes between monocytes/monocyte derived cells from αCSF-1- and isotype control MAb-treated mice.

Supplemental Figure 10



Supplemental Figure 10. Monocytes from the CNS of αCSF-1 MAb-treated mice with EAE have a transcriptionally distinct phenotype. EAE was induced in C57BL/6J mice by immunization with MOG_{35-55}. Mice were treated with 200 μg/day of αCSF-1 MAb or isotype IgG1 control MAb (n=3/group) from day 11 to 16 p.i. and sacrificed on day 17 p.i. **A)** Numbers of $CD45^{Hi}CD11b^{+}Ly6G^{-}Ly6C^{+}$ monocytes. **B)** Flow cytometry plots showing expression of Ly6C and Ly6G among $CD45^{+}CD11b^{+}$ cells. **C)** Heatmap showing differentially expressed transcripts in monocytes from αCSF-1- and isotype MAb-treated mice. Criteria for inclusion in heatmap were expression in all samples, with a p-adjusted value < 0.05 and a $\log_2$ fold change greater/less than ±1. **D)** Volcano plot showing relative expression of transcripts detected in samples from αCSF-1- and isotype MAb-treated mice. **E)** Gene ontology and **F)** KEGG pathway terms that were significantly enriched ($p<0.05$) and ranked by percentage of differentially expressed genes (p-adj<0.01). **G)** Heatmap of z-score- normalized TPM data from differentially expressed genes detected from the PI3K-Akt signaling pathway KEGG term.

Supplemental Figure 11



Supplemental Figure 11. High degree of chimerism in treosulfan-induced BM chimera mice; and reduction of moDCs in CSF1R-iKO/WT mixed BM chimeras. A) Western blot showing protein level of CSF-1R in cell lysates of whole spleen, draining lymph nodes, and CNS mononuclear cells 5 days after 5-day treatment with tamoxifen. Relative expression, shown in the right panel, was calculated by densitometry of CSF-1R signal relative to β-actin signal, then normalized to WT expression. Each bar represents pooled lysate from 5 mice, with equal numbers of cells from each mouse pooled together. **B)** EAE in tamoxifen-treated CSF1R-iKO (n=6) and UBC-CreERT2 (n=3) mice. Significance was determined by two-way ANOVA. **C)** WT CD45.2$^+$ recipient mice were treated with treosulfan and rested for 72 h, then reconstituted with 1x10^7 BM cells from CD45.1$^+$ mice. After 32 days, blood was collected via tail vein and reconstitution efficiency determined. Left panel shows flow cytometry plot of CD45.1 and CD45.2 expression in PBMCs. Right panel shows quantification of reconstitution efficiency. WT CD45.2 (grey bar) represents blood from naïve WT CD45.2$^+$ mice. **D)** Quantification of CD45$^+$SalI1$^-$CD11b$^+$Ly6G$^-$CD11c$^+$Ly6C$^+$MHCII$^+$ moDCs in mixed BM chimeras. Left panel shows moDCs in the CNS from mice reconstituted with a 1:1 mixture of WT and CSF1R-iKO BM cells. Right panel shows moDCs from mice reconstituted with a 1:1 mixture of CD45.1 WT and CD45.2 WT BM cells. moDCs derived from CD45.1 and CD45.2 immune cells in the CNS were compared. Significance was determined by paired *t* test. **E)** Schematic showing procedure for experiment described in Figure 6E. **F)** Schematic showing procedure for experiment described in Figure 6F.