

Supplemental Materials and Methods.

T cell proliferation and cytokine assays. T cells were isolated from draining LN or spleens of MOG-immunized mice via a CD3⁺ column (R&D Systems). CD3⁺ T cell purity was >80% for spleen preparations and >98% for LN, as determined by flow cytometric analysis (data not shown). For preparation of antigen presenting cells (APC), red blood cells were removed from the spleens of naive WT or CMKLR1 KO mice via hypotonic lysis. Spleen cells were then resuspended at 5×10^7 cells/ml in PBS, followed by incubation with 50 µg/ml mitomycin C (MMC; Sigma) for 20 min at 37°C. MMC-treated splenocytes were washed extensively and resuspended in RPMI with supplements. T cells and APC were plated in 96-well flat-bottom plates; each well contained 2.5×10^5 APC mixed with 5×10^4 T cells. MOG₃₅₋₅₅ was added at 25 µg/ml and cells were incubated for 72 h at 37°C, 8% CO₂. For assessment of proliferation, [³H]-thymidine was added for the last 18-24 h of culture, and thymidine incorporation in triplicate wells was assessed using a beta-plate scintillation counter. For cytokine measurements, culture supernatants were harvested at 72 h; levels of IFN γ (BD Pharmingen) and IL-17 (eBioscience) in triplicate wells were determined by sandwich ELISA according to the manufacturer's instructions.

Peritoneal macrophage responses. Peritoneal lavage cells (PLCs) were collected by lavage of the peritoneal cavity with 10 ml sterile HBSS containing 2% FBS. Harvested cells were washed and resuspended in DMEM containing 10% FBS, penicillin/streptomycin, L-glutamine, sodium pyruvate and nonessential amino acids. Cells were plated in 24-well tissue culture plates at 5×10^5 cells per well and allowed to adhere for 2 h at 37°C. Non-adherent cells were washed away with PBS, and adherent cells were stimulated with 1 µg/ml lipopolysaccharide (LPS; Sigma) plus 10 ng/ml recombinant murine IFN γ (eBioscience). Culture supernatants were harvested

after 24 h, and levels of IL-6 and TNF were assayed by ELISA. Where indicated, mice were administered 1.0 ml of 3% thioglycollate (Sigma) via i.p. injection 72 h prior to PLC harvest.

Real-time quantitative PCR. Mice were perfused, and spinal cord RNA was extracted using a Stratagene RNA miniprep kit per the supplier's instructions. Gene expression was determined by quantitative PCR (QPCR) using an Applied Biosystems 7900HT real-time PCR instrument equipped with a 384-well reaction block. 100-200 ng total RNA was used as template for cDNA synthesis using MMLV Reverse Transcriptase (Applied Biosystems) with oligo dT primers (Invitrogen) according to the supplier's instructions. The cDNA was diluted 1:12 and amplified by quantitative PCR in triplicate wells using 10 pmols of gene specific primers in a total volume of 10 μ l with Power SYBR Green QPCR Master Mix (Applied Biosystems), according to manufacturer's instructions. Relative gene expression normalized to cyclophilin A (*cycA*) was calculated; relative gene expression values (multiplied by 10^4 to simplify data presentation) are displayed. Primer sequences were as follows: *cycA* forward, 5'-GAGCTGTTTGCAGACAAAGTTC-3'; *cycA* reverse, 5'-CCCTGGCACATGAATCCTGG-3'; *chemerin* forward, 5'-TACAGGTGGCTCTGGAGGAGTTC-3'; *chemerin* reverse, 5'-CTTCTCCGTTTGGTTTGATTG-3'; *cmklr1* forward, 5'-CGGTCTTCCTGGTGGTGA-3'; *cmklr1* reverse: 5'-GCACATGGCCTTCCCGAA-3'.

Evaluation of anti-CMCLR1 mAbs by flow cytometry. Spleen cells or PLCs were harvested from naïve C57BL/6 mice, and stained with rat anti-mouse CMCLR1 mAb BZ194 or mouse anti-mouse CMCLR1 mAb BZ186 and their respective isotype controls. PE-conjugated goat anti-rat IgG (BD Pharmingen) was used to detect BZ194; PE-labeled rat anti-mouse IgG1 was used to detect BZ186. Cells were then stained with fluorophore-labeled monoclonal antibodies to identify the indicated leukocyte subsets and data were acquired on a flow cytometer. CMCLR1

expression was analyzed on NK cells (NK1.1⁺CD3⁻), T cells (CD3⁺NK1.1⁻), B cells (CD19⁺) and DC (CD3⁻CD19⁻NK1.1⁻CD11c⁺) isolated from the spleen; macrophages (F4/80⁺CD11b⁺) were taken from the peritoneal cavity. The anti-NK1.1 mAb (PK136) was from eBioscience.

Supplemental Figure Legends.

Supplemental Figure 1. Proliferation and cytokine production by CMKLR1 KO T cells.

CMKLR1 KO or WT mice were immunized with MOG₃₅₋₅₅ emulsified in CFA. Ten days later, CD3⁺ T cells were isolated from spleen or draining LN, followed by co-incubation with MMC-treated WT or CMKLR1 KO splenocytes. [All possible T cell:APC combinations were utilized: i) WT:WT; ii) WT:KO; iii) KO:WT; iv) KO:KO.] MOG₃₅₋₅₅ was added at 25 µg/ml. (A) After 72 h of stimulation, proliferation of LN (left panel) or splenic T cells (right panel) was assessed by ³H-thymidine incorporation assay. Data are presented as a stimulation index; bars represent s.e.m. (B) Culture supernatants were collected after 72 h, and levels of the indicated cytokines produced by LN (left panels) or splenic T cells (right panels) were measured by ELISA.

Asterisks in panel (A) indicate statistically significant differences ($P < 0.05$) as follows: *

WT:WT vs. KO:KO; ** KO:WT vs. KO:KO; and *** WT:WT vs. KO:WT, as determined by analysis of variance (ANOVA), followed by Bonferroni multiple comparisons post test. Data shown are representative of two independent experiments with similar results.

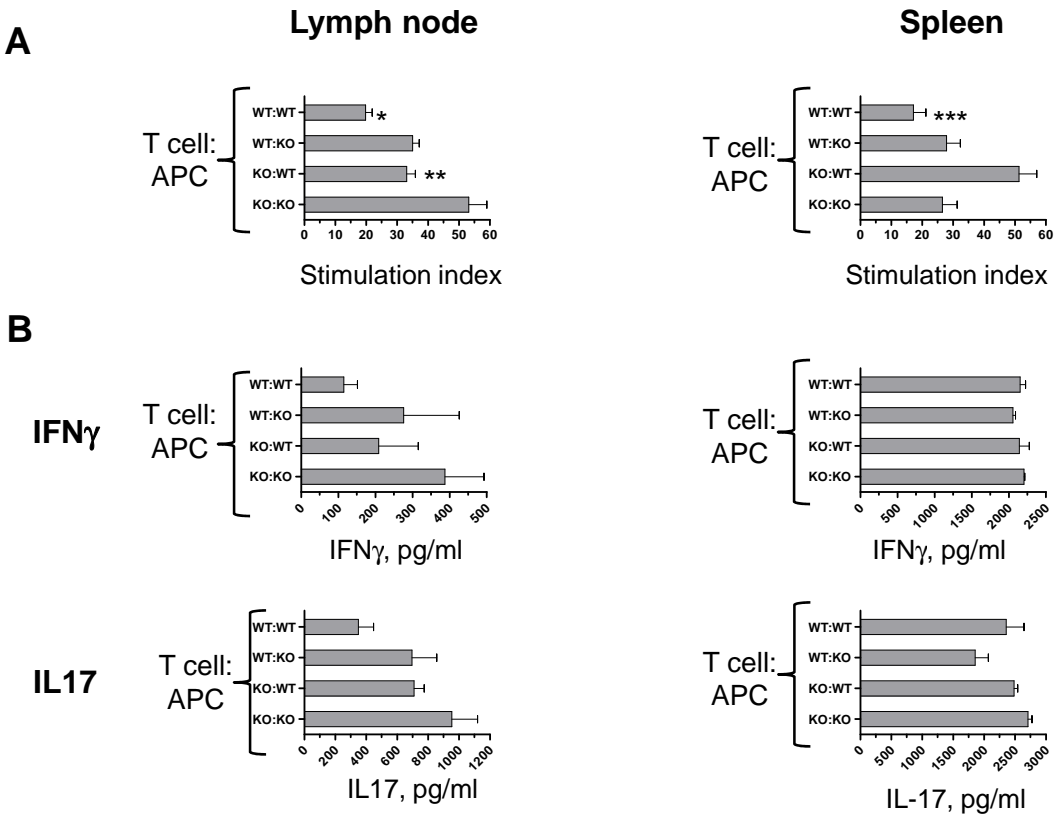
Supplemental Figure 2. Peritoneal macrophage responses in CMKLR1 KO mice. (A)

Peritoneal lavage cells (PLCs) were harvested from naïve WT or CMKLR1 KO mice. After allowing PLCs to adhere for 2 h at 37 C, non-adherent cells were washed away and adherent cells were stimulated with LPS/IFN γ for 24 h. Levels of TNF (top panel) and IL-6 (bottom panel) in culture supernatants were measured by ELISA. (B) WT and CMKLR1 KO were given a single i.p. injection of thioglycollate. Mice were killed 72 h later and PLCs were harvested and processed as described in (A). Levels of TNF and IL-6 were measured by ELISA. * $P < 0.05$, as determined by Student's *t* test. Data shown are representative of two independent experiments with similar results.

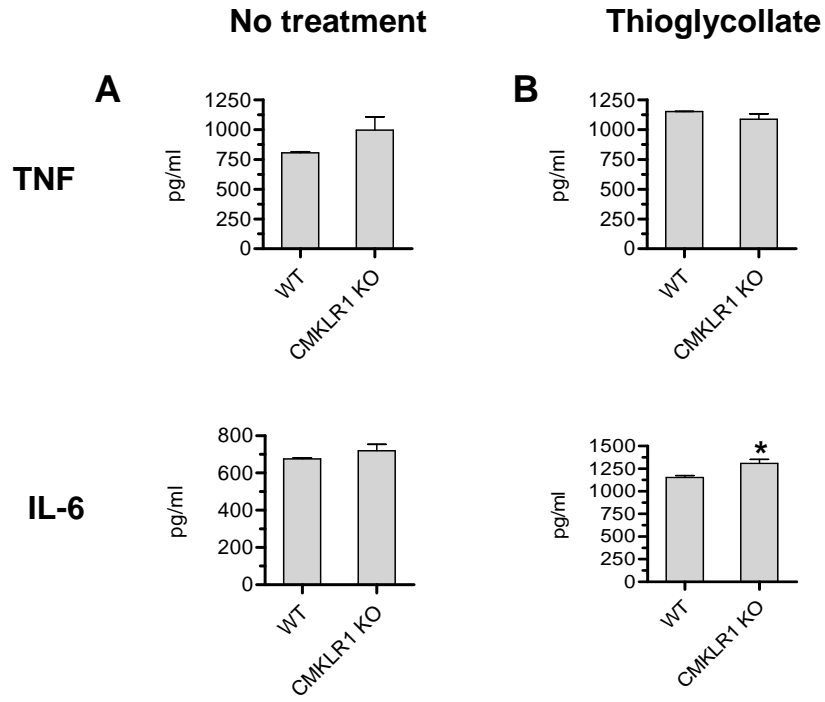
Supplemental Figure 3. Chemerin transcripts are up-regulated in the CNS of mice with EAE. Spinal cord RNA was extracted from naïve mice (n=9), or from mice induced to develop EAE at day 13 p.i. (n=8). Relative chemerin RNA expression (normalized to cycA) was assessed by real-time QPCR as described in Supplemental Materials and Methods. Values are presented as mean \pm s.e.m. * $P < 0.05$, as determined by Student's *t* test.

Supplemental Figure 4. Staining of mouse leukocytes with anti-CMKLR1 mAbs. Spleen cells or PLCs were harvested from naïve C57BL/6 mice, and stained with rat anti-mouse CMKLR1 mAb BZ194 (A) or mouse anti-mouse CMKLR1 mAb BZ186 (B) and their respective isotype controls. PE-conjugated goat anti-rat IgG (BD Pharmingen) was used to detect BZ194; PE-labeled rat anti-mouse IgG1 was used to detect BZ186. Cells were then stained with fluorophore-labeled monoclonal antibodies to identify the indicated leukocyte subsets and data were acquired on a flow cytometer. CMKLR1 expression was analyzed on NK cell, T cells, B cells and DC isolated from the spleen; macrophages were taken from the peritoneal cavity. Filled histograms represent BZ332 (top panels) or DREG200 (bottom panels) isotype control staining. Open histograms indicate staining with BZ194 (top panels) or BZ186 (bottom panels).

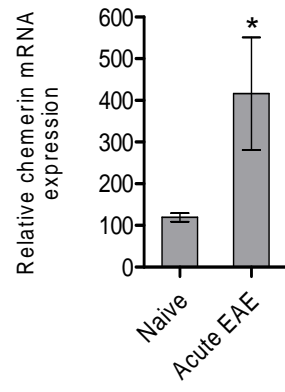
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4

