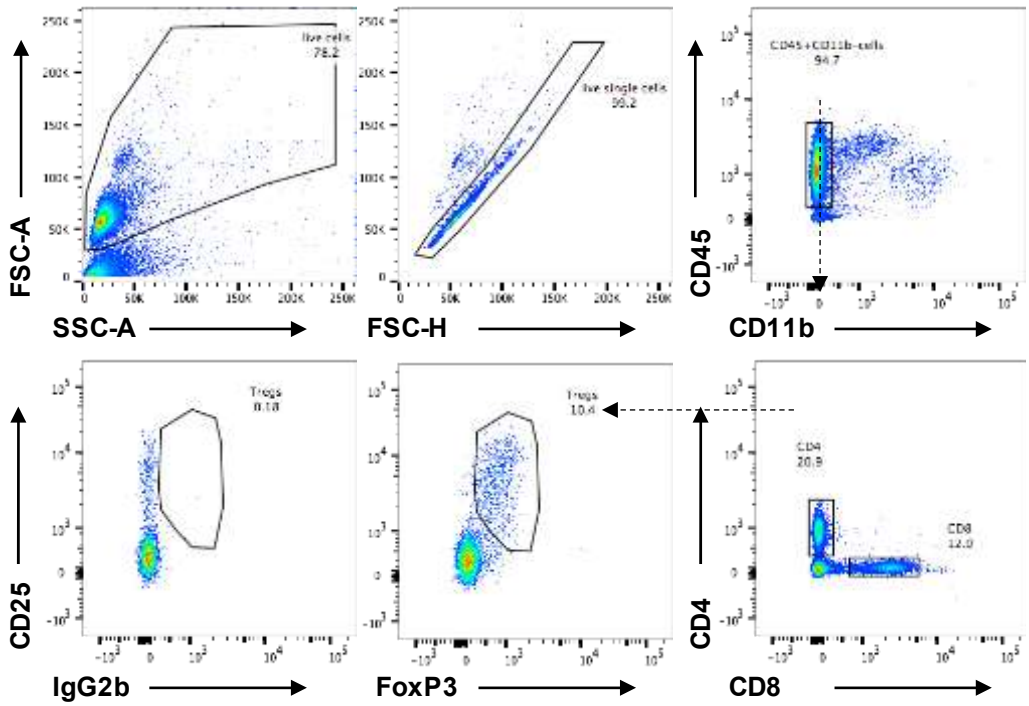
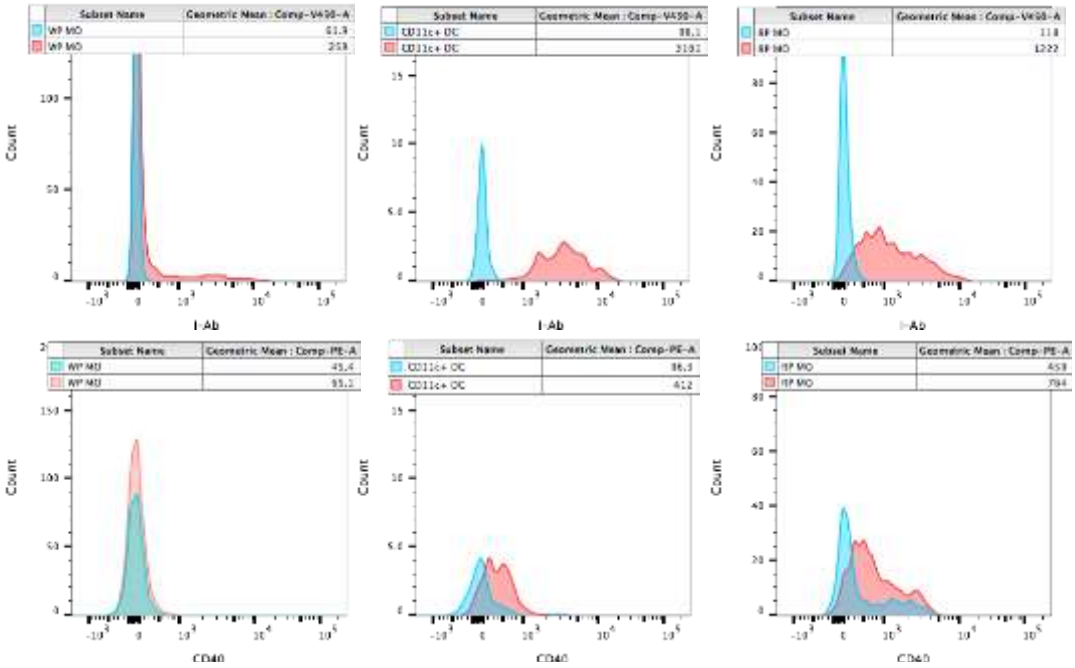
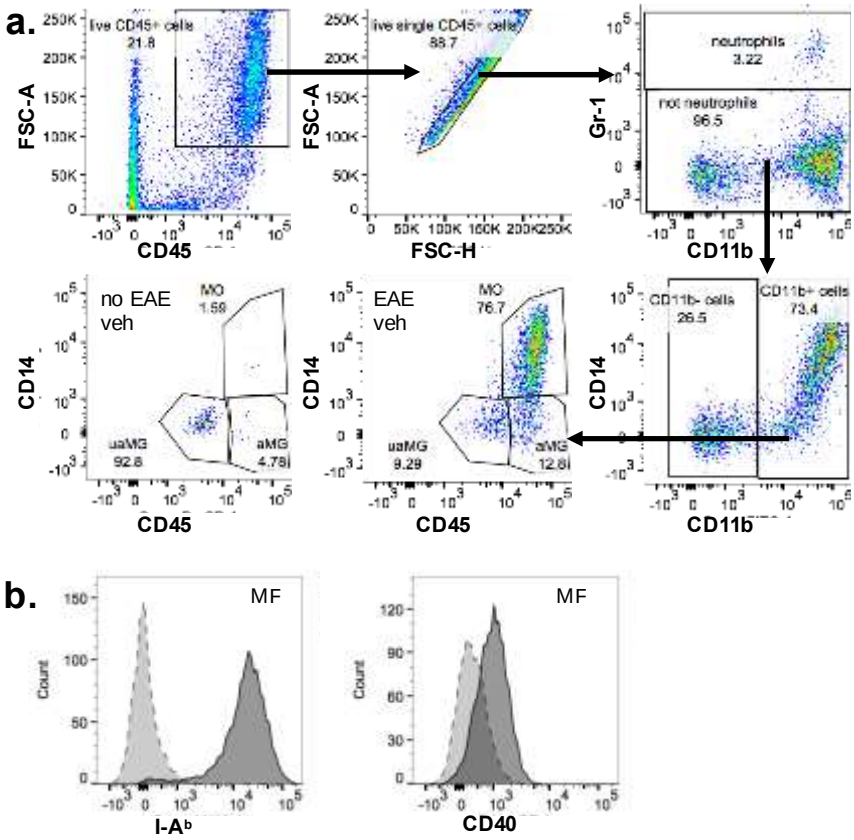
Supplementary Material



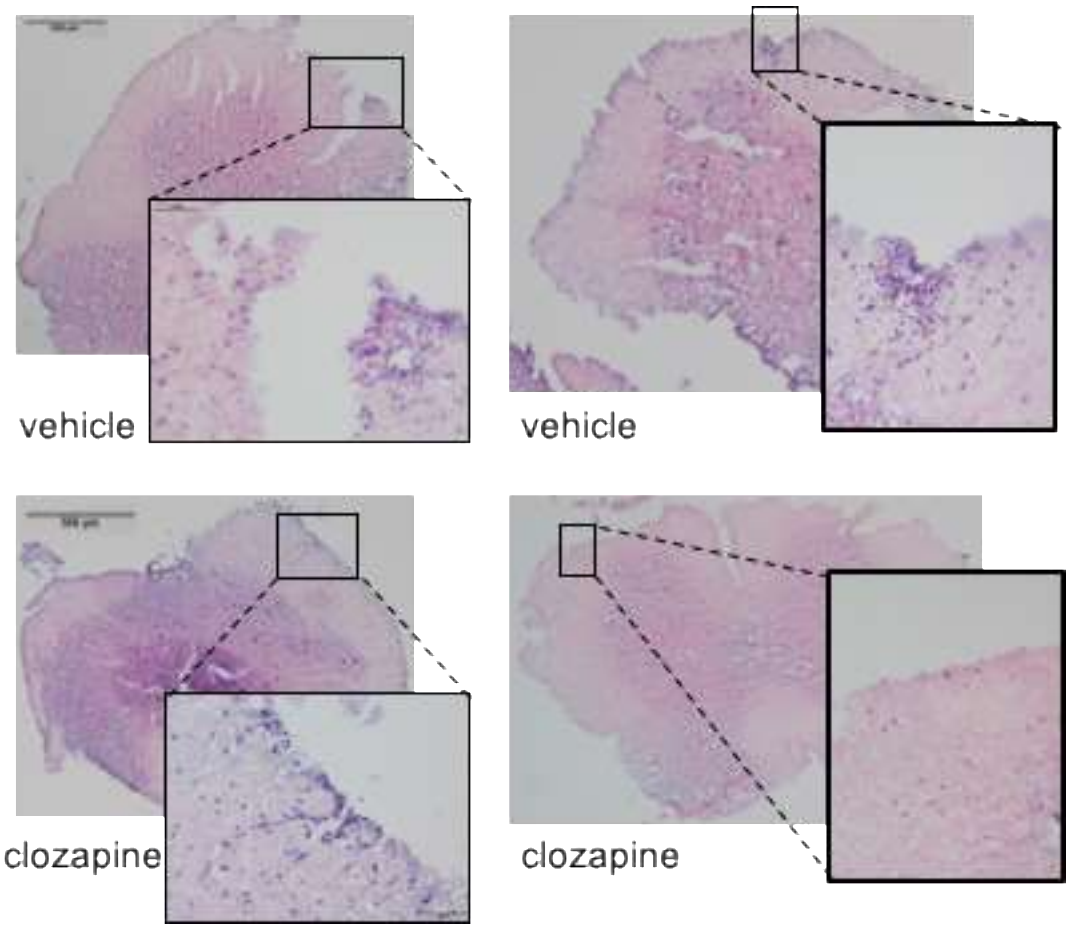
Supplementary Figure 1: Gating strategy for T cell populations. C57BL/6 mice were immunized and treated with clozapine (60 mg/kg/day) or vehicle as in Figure 1a. Fifteen days post-immunization, splenocytes were isolated and analyzed by flow cytometry. Shown are representative flow plots illustrating the gating strategy for CD4 T cells, CD8 T cells, and $CD25^{+}FoxP3^{+}CD4^{+}$ T cells (Tregs).



Supplementary Figure 2: Expression of I-Ab and CD40 on splenic myeloid subsets. C57BL/6 mice were immunized and treated with clozapine (60 mg/kg/day) or vehicle as in Figure 1a. Fifteen days post-immunization, splenocytes were isolated and analyzed by flow cytometry. Splenic myeloid populations were identified using the gating strategy in Figure 3. Shown are representative histograms illustrating the expression of I-A^b (top row) and CD40 (bottom row) on white pulp macrophages (WPMO; left), $CD11c^+$ DC (middle), and red pulp macrophages (RPMO; right). Red = I-A^b/CD40 Blue = isotype control. GeoMFI is listed in the table at the top of each graph.



Supplementary Figure 3: Gating strategy for spinal cord myeloid cell infiltrates. C57BL/6 mice were immunized and treated with clozapine (60 mg/kg/day) or vehicle as in Figure 1a. Fifteen days post-immunization, cells were isolated from spinal cords and analyzed by flow cytometry. (a) Gating strategy for all myeloid cells. (b) Expression of I-A^b and CD40 on gated macrophages (Mϕ). Specific antibodies for I-A^b and CD40 (dark grey) and isotype control antibodies (light grey) demonstrate specific detection of each marker. Shown are representative flow plots and histograms.



Supplementary Figure 4: Spinal cord lesion analysis. C57BL/6 mice were immunized and treated with clozapine (60 mg/kg/day) or vehicle as in Figure 1a. Fifteen days post-immunization, spinal cords were isolated after transcardially perfusion with PBS. Following post-fixation in 4% PFA in PBS the tissue was mounted with Jung tissue freezing medium (Leica Biosystems, Nussloch, Germany) and 30 µM sections were assessed for lesion size and number, and for inflammation accumulation by hematoxylin and eosin-staining. Shown are representative sections from 2 vehicle-treated (top) and 2 clozapine-treated (bottom) animals.

Supplementary Methods:

Flow cytometric analysis

Splenocytes and spinal cord mononuclear cells were characterized for immune phenotypes and activation markers using the following antibodies: rat anti-CD45-V450 or PECy5, rat anti-CD4-BV510 or PE, rat anti-CD11b-BB515, rat anti-Gr-1-APC-Cy7, rat anti-FoxP3-PE, and rat anti-CD40-PE from BD Biosciences; rat anti-CD8-BV421 or PerCP-Cy5.5, rat anti-CD14-PE-Cy7, hamster anti-CD11c-APC, rat anti-CD25APC or AF488, rat anti-FoxP3-AF647, and rat anti-I-A/I-E-BV421 from BioLegend (San Diego, CA, USA); and rat anti-F4/80-biotin from Serotec (Kidlington, UK). Streptavidin-V500 (BD Bioscience) was used to detect biotinylated antibodies. Each antibody was matched to its specific, fluorescently-labelled isotype control according to the manufacturer's recommendations. All flow data was collected on a FACSCanto II (Becton Dickinson, Franklin Lakes, NJ, USA) and analysed using FlowJo v6.1 & 10.1r7 (Tree Star Inc., Ashland, OR, USA).

Quantification of serum clozapine and n-desmethyl clozapine (norclozapine) levels

Rapid detection of clozapine and norclozapine by liquid chromatography/tandem mass spectrometry (LCMS) technology has been previously described.[25] In brief, serum samples were isolated from blood collected by cardiac puncture and centrifuged at 7800 x g for 10 min at 4˚C. Quetiapine, as an internal standard (IS), was added to each serum sample at a final concentration of 500 ng/mL in a total volume of 25 µL. An equal volume of HPLC-grade acetonitrile was then added to each preparation, vortexed for 15 sec, and separated by centrifugation at 13,000 rcf for 5 min. The supernatant was then transferred to a fresh tube where 20 µL was loaded into an HPLC vial containing a 200 µL glass insert (Thermo Scientific, Massachusetts, USA). Clozapine standards (1000 ng/mL – 15ng/mL) were prepared as above in serum from untreated animals.

LCMS data was obtained using an Agilent 6530 Q-TOF instrument operating in positive ion mode with a JetStream electrospray ionization source. The ion source was run with a drying gas temperature of 275 °C at a flow of 9 L/min and a nebuliser pressure of 40 psi. The sheath gas flow was 10 L/min at 300 °C. The electrospray capillary was set to 4000 V, with a nozzle voltage of 500 V and the fragmentor at 100 V. Two scans per second were acquired between m/z 100 and 3200 with constant infusion of reference ions to maintain mass accuracy. Samples were injected (0.5 µL) onto a Phenomenex Eclipse Plus C18 column (30 x 2.1 mm, 3.5 µm particle sizes) using 0.05 mM ammonium formate in deionised water and acetonitrile as running solvents, at 0.4 mL/min flow rate at 35 °C. The following solvent gradient was used: Initial: 5% acetonitrile held for 0.5 min; linear ramp to 100% ACN over 13.5 min, held for 1 min; linear ramp to 5% acetonitrile over 0.1 min, held till 18 min. Data was acquired and processed using Agilent Technologies Mass Hunter software.