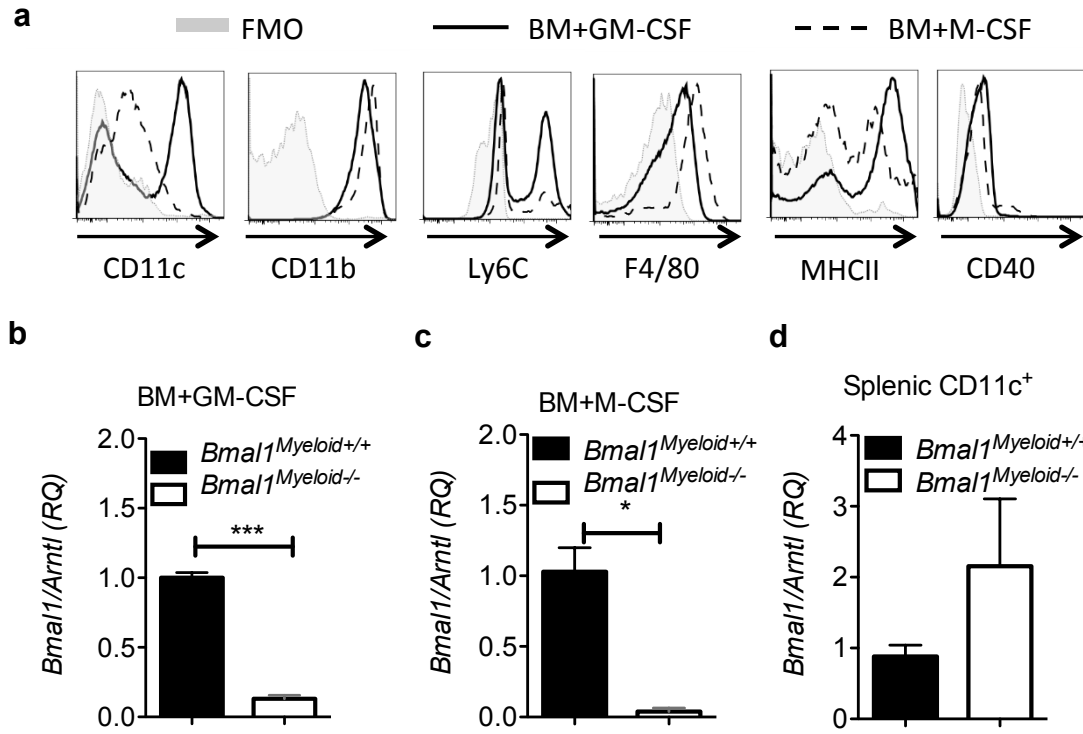


Supplementary Figure 1

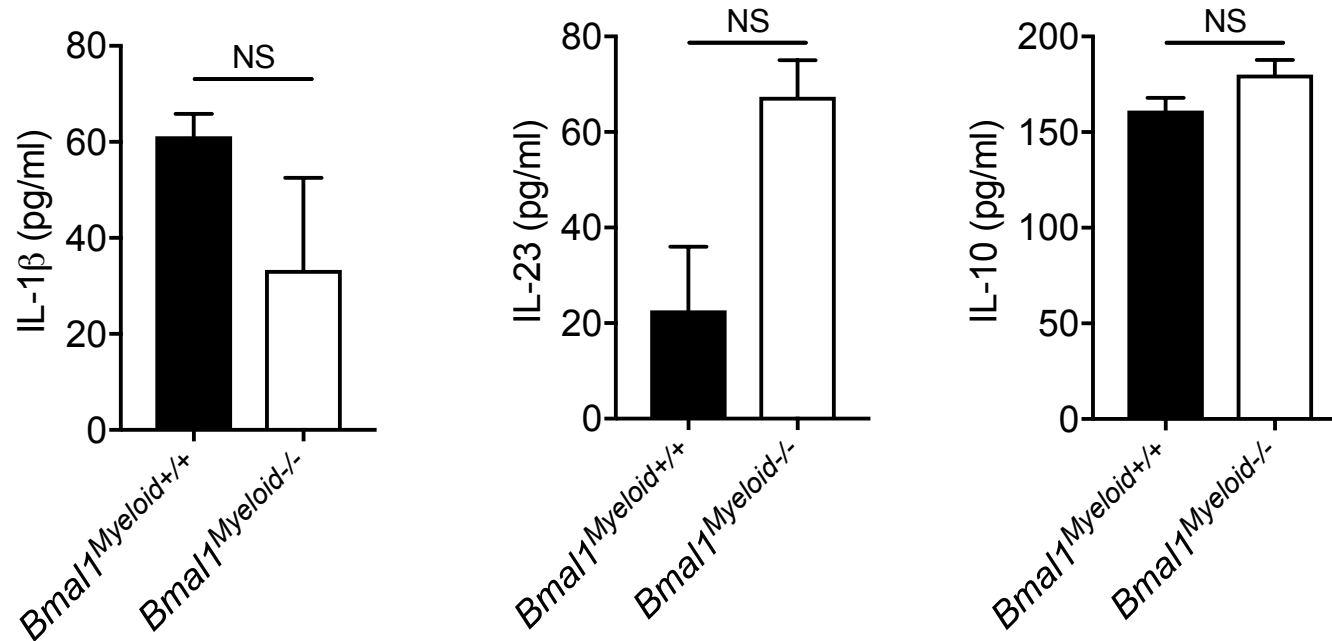


Bone marrow expanded cells from *Bmal1*^{Myeloid-/-} mice are devoid of *Bmal1*.

Bone marrow (BM) cells from C57BL/6 mice (a) or *Bmal1*^{Myeloid+/+} and *Bmal1*^{Myeloid-/-} mice (b-d) was cultured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) (20 ng/ml) or macrophage colony-stimulating factor (M-CSF) (100 ng/ml) for 6 d. (a) FACS surface staining for CD11c, CD11b, Ly6C, F4/80, MHCII or CD40. (b and c) Cells were harvested into Trizol and RNA was extracted. RT-PCR for *Bmal1* was performed on each sample, relative to *Bmal1*^{Myeloid+/+} cells.

(d) CD11c⁺ cells were FACS-sorted from the spleens of naïve *Bmal1*^{Myeloid+/+} and *Bmal1*^{Myeloid-/-} mice. RNA was extracted by Trizol from sorted cells and was tested by RT-PCR for *Bmal1* (n=3). FMO = Fluorescence minus one. RQ = Relative Quantification. Presented as means +/- standard error of the mean (SEM). Statistics were performed by Mann-Whitney U test. *, p<0.05; ***p<0.001.

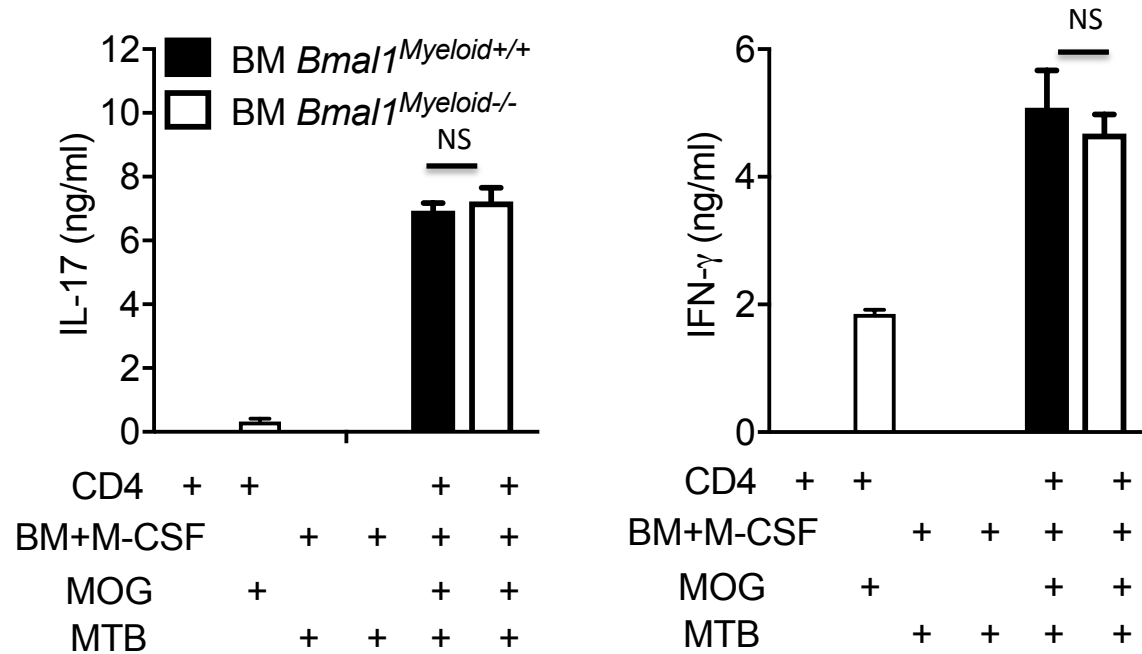
Supplementary Figure 2



Loss of *Bmal1* from bone marrow cells cultured with M-CSF does not affect MTB driven pro-inflammatory responses.

Bone marrow (BM) cells isolated from *Bmal1^{Myeloid}+/+* or *Bmal1^{Myeloid}-/-* mice were cultured with macrophage colony-stimulating factor (M-CSF) (100 ng/ml). After 6 days cells were harvested and stimulated with *Mycobacterium tuberculosis* (MTB) (100 µg/ml). Supernatants were removed at 24 h and tested by ELISA for cytokine production (n=3). Presented as means +/- standard error of the mean (SEM). Statistics were performed by Mann-Whitney U test.

Supplementary Figure 3

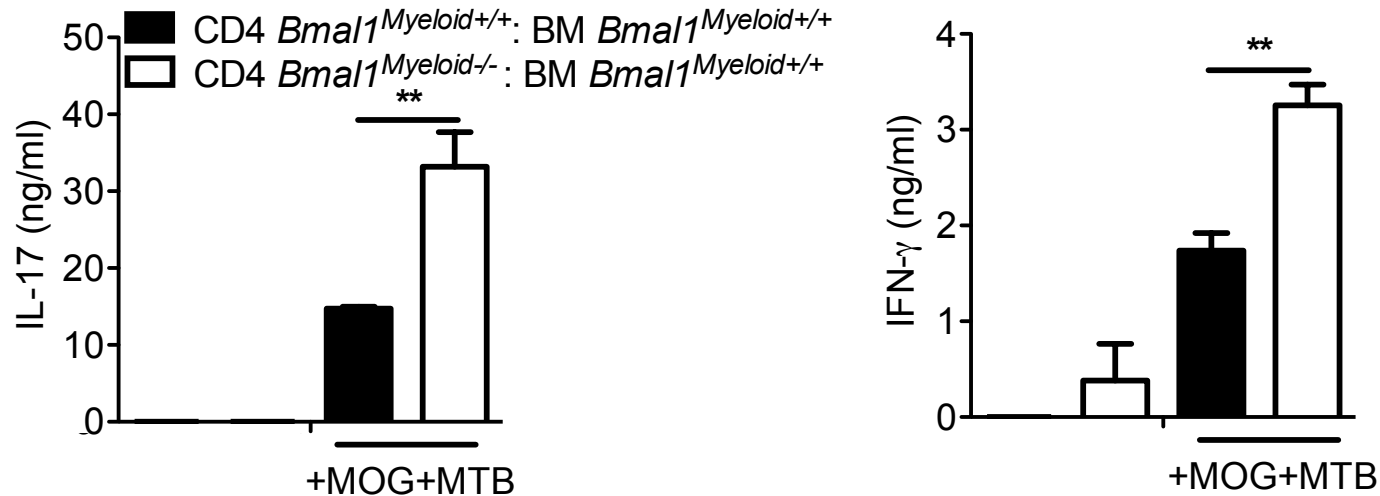


Loss of *Bmal1* from bone marrow cells cultured with M-CSF does not affect antigen-specific pro-inflammatory T cell responses.

Bone marrow (BM) cells from *Bmal1*^{Myeloid+/+} and *Bmal1*^{Myeloid-/-} mice were cultured in the presence of macrophage colony-stimulating factor (M-CSF). After 6 d cells were harvested and incubated with myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) (25 µg/ml) + with *Mycobacterium tuberculosis* (MTB) (100 µg/ml) or with medium for 3 h prior to the addition of MACS purified CD4 T cells isolated from the draining lymph nodes of 7 d MOG₃₅₋₅₅+ complete Freund's adjuvant (CFA) immunised *Bmal1*^{Myeloid+/+}.

After 72 h of co-culture supernatants were removed and tested for IL-17 and IFN-γ by ELISA (n=3). Presented as means +/- standard error of the mean (SEM). Statistics were performed by Mann-Whitney U test.

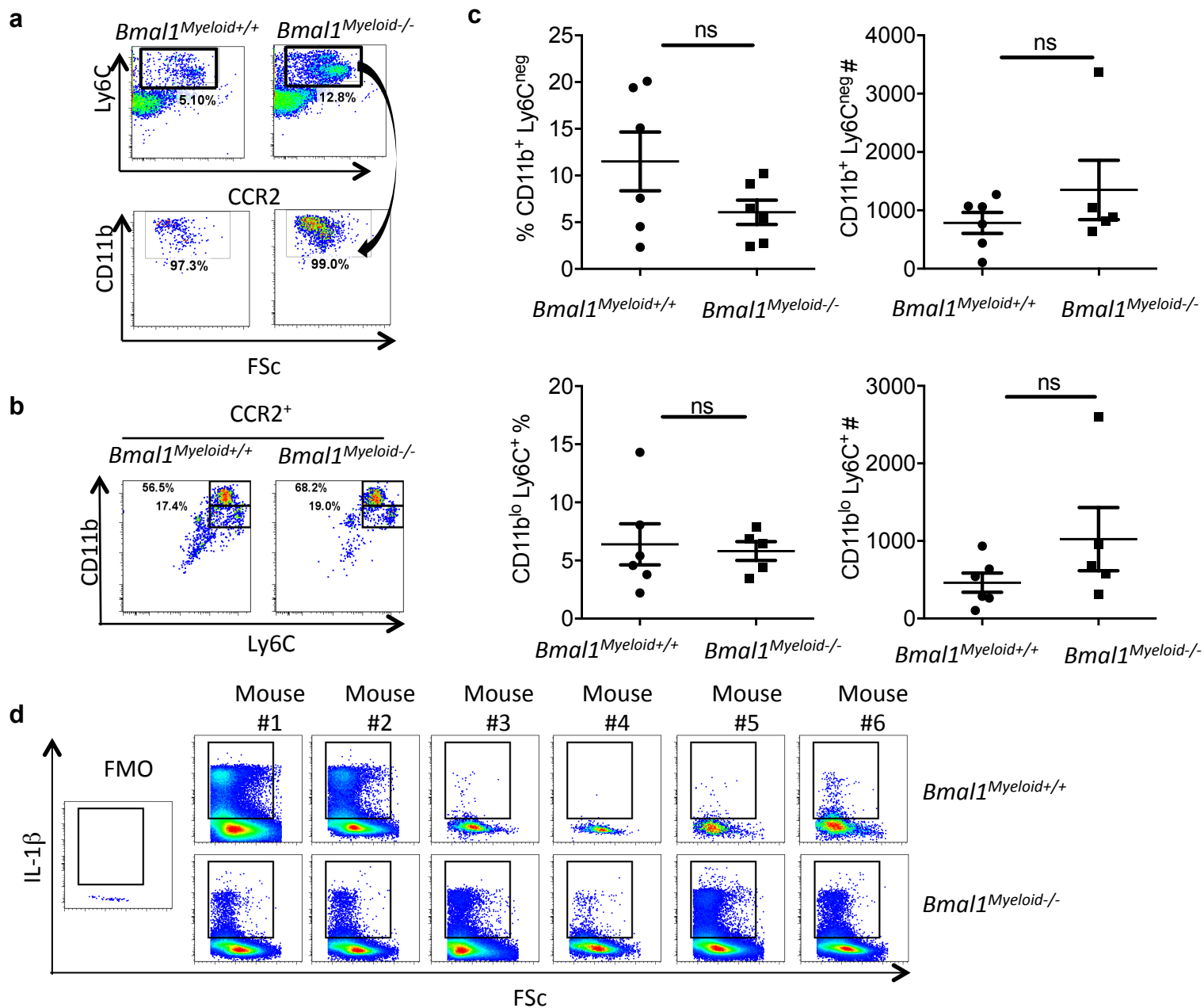
Supplementary Figure 4



T cells isolated from immunized mice lacking myeloid *Bmal1* display enhanced antigen specific pro-inflammatory responses *ex vivo*.

Bone marrow (BM) cells from *Bmal1^{Myeloid+/+}* was cultured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF). After 6 d cells were harvested and incubated with myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) (25 µg/ml) + *Mycobacterium tuberculosis* (MTB) (100 µg/ml) or with medium for 3 h prior to the addition of MACS purified CD4 T cells isolated from the draining lymph nodes of 7 d MOG₃₅₋₅₅+ complete Freund's adjuvant (CFA) immunised *Bmal1^{Myeloid+/+}* or *Bmal1^{Myeloid-/-}* mice. After 72 h of co-culture supernatants were removed and tested for IL-17 and IFN-γ by ELISA (n=3). Presented as means +/- standard error of the mean (SEM). Statistics performed by Mann-Whitney U test. **, p<0.01.

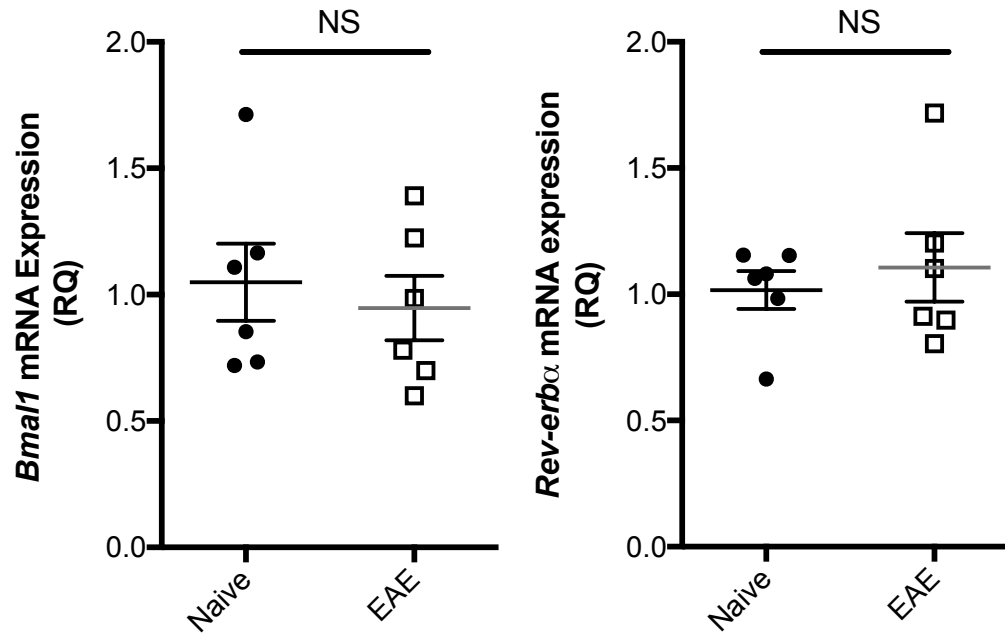
Supplementary Figure 5



Inflammatory populations associated with disease progression are enhanced in *Bmal1*^{Myeloid-/-} mice at the height of EAE.

Mice were immunised with myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) + complete Freund's adjuvant (CFA), and injected with pertussis toxin (PT) (125 ng/mouse) on d 0 and d 2. (a-c) 14 d post immunisation infiltrating mononuclear cells in the spinal cords of mice and were stained on their surface for a CD11b, Ly6C and CCR2 expression. (a) Percentage of CD11b⁺ cells gating on Ly6C⁺CCR2⁺ cells the percentage of CD11b⁺ cells v FSc. (b) Percentage of CD11b^{hi}Ly6C⁺ of CCR2⁺ cells were examined. (c) Numbers and percentages of CD11b⁺ cells were examined (n=5-6). (d) D 10 post immunisation for EAE, IL-1 β expression was determined in mononuclear cells isolated from the brains of 6 individual *Bmal1*^{Myeloid+/+} or *Bmal1*^{Myeloid-/-} mice, gating on live CD45⁺CD11b⁺Ly6C⁺ cells. Presented as means +/- standard error of the mean (SEM). Statistics performed by Mann-Whitney U test.

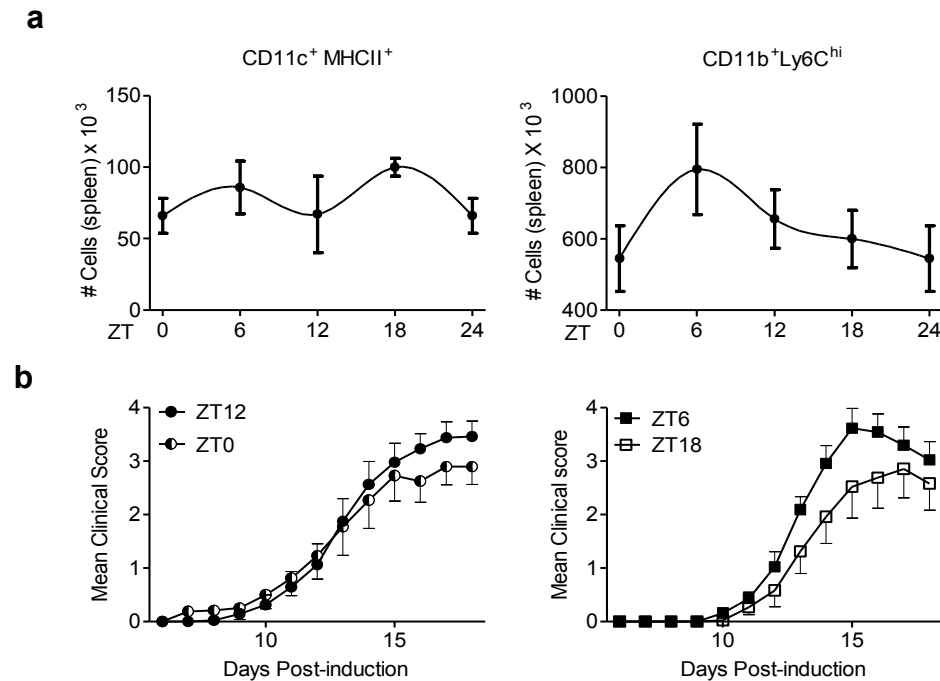
Supplementary Figure 6



***Bmal1* and *Reverba* are not affected under systemic inflammation in spinal cords.**

Spinal cords were removed from 14 d complete Freund's adjuvant (CFA) and pertussis toxin (PT)-immunised or naïve female C57BL/6 mice (no myelin oligodendrocyte glycoprotein (MOG) was used). RT-PCR analysis of *Bmal1* and *Rev-Erba* mRNA of isolated spinal cords (n=6). RQ = Relative Quantification. Presented as means +/- standard error of the mean (SEM). Statistics performed by Mann-Whitney U test.

Supplementary Figure 7

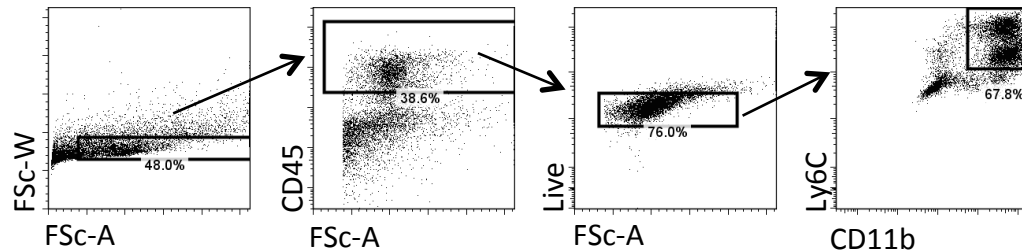


Time of day dependent alterations in the spleen and induction of Experimental Autoimmune Encephalomyelitis (EAE)

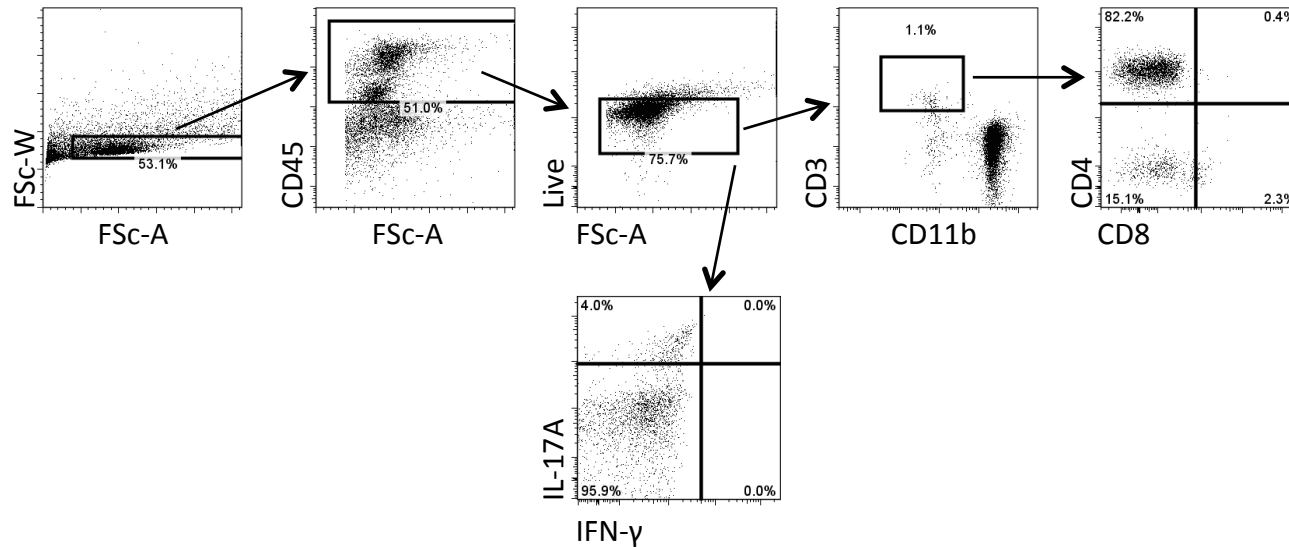
(a) CD11c⁺MHCII⁺ and CD11b⁺Ly6C^{hi} populations were FACS sorted from spleens of 5 individual C57BL/6 mice at ZT0, 6, 12 or 18. ZT0 has been double plotted to continue trend. Total numbers of cells sorted were recorded for each population (n=5-6). (b) Time of day was adjusted in C57BL/6 female mice (Harlan, UK), using a light cabinet so that both ZT6 and ZT18 corresponded to 3pm and ZT0 and ZT12 corresponded to 9am. Mice were immunised with myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) + complete Freund's adjuvant (CFA) and pertussis toxin (PT) (200 ng/mouse), which had been emulsified 1 week in advance at 4pm, and mice were given a second dose of PT at 4pm or 9am on d 2. Mice were scored daily for the disease score. Data presented as means $\pm$ standard error of the mean (SEM) of 12 mice per group.

Supplementary Figure 8

a



b



Gating strategies used for flow cytometry analysis of CD11b⁺, CD4⁺, CD8⁺ cells and IFN-γ⁺ and IL-17⁺-producing cells.

(a) Gating strategy used to gate on CD11b⁺ cells as presented on Figure 3. The same type of gating strategy was used in Figures 1, 2 and 6 (b) Gating strategy used to gate on CD4⁺, CD8⁺ cells and IFN-γ⁺ and IL-17⁺-producing cells as presented in Figure 4.