Supporting Information

Selective endocannabinoid reuptake inhibitor WOBE437 reduces disease progression in a mouse model of multiple sclerosis

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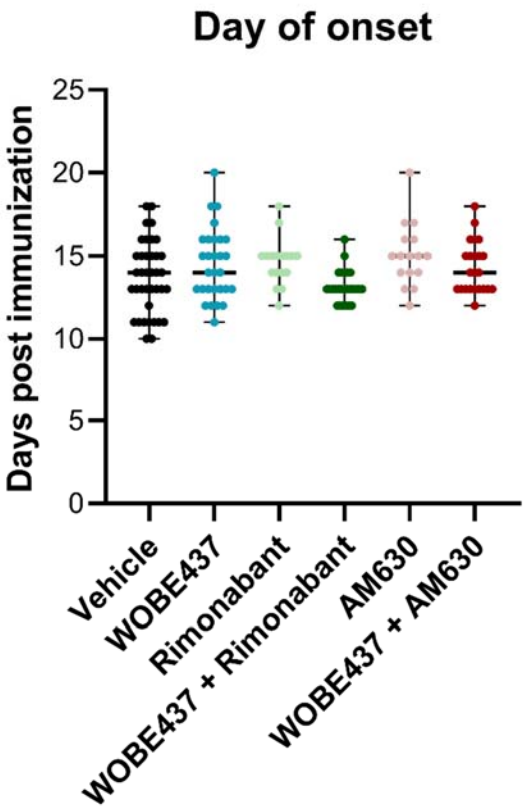
ASSOCIATED CONTENT

Table S-1. Flow cytometry antibodies

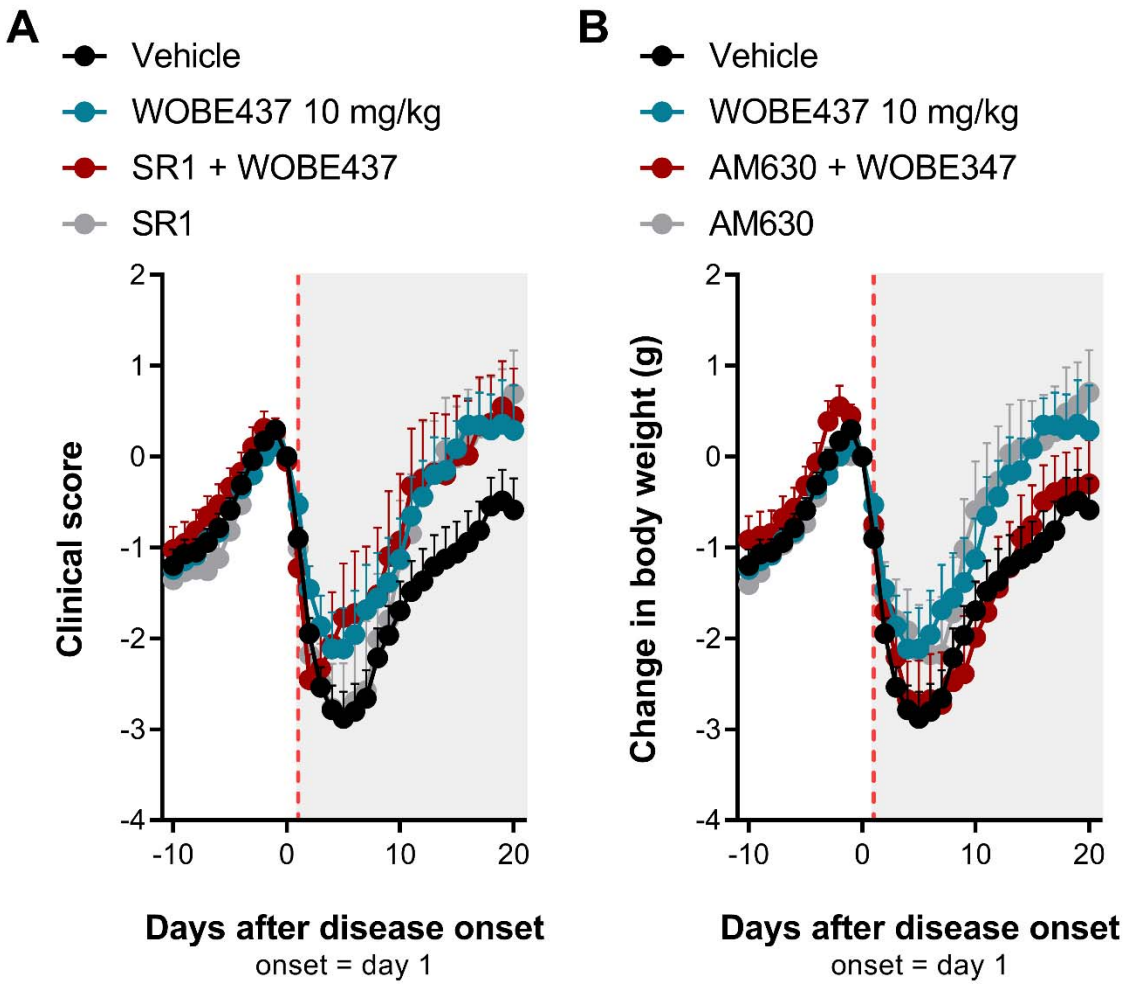
Antigen	Clone	Company	Isotype control
CD45 PE/Cy7	30-F11	LSBio	rat IgG2b PE/Cy7
CD4 Alexa700	RM4-5	Biolegend	rat IgG2b Alexa700
CD8 PE	53-6.7	Biolegend	rat IgG2a PE
IL-17 PE-eFluor610	eBio17B7	eBioscience	rat IgG2a eFlour610
IFNγ eFluor450	XMG1.2	ebioscience	rat IgG1 eFluor450
IL-10 APC	JES5-16E3	Biolegend	rat IgG1 APC
GM-CSF PerCP/Cy5.5	MP1-22E9	Biolegend	rat IgG2a PerCP/Cy5.5

Figure S-1. Distribution of the day of onset showed in all the mice included in the study.



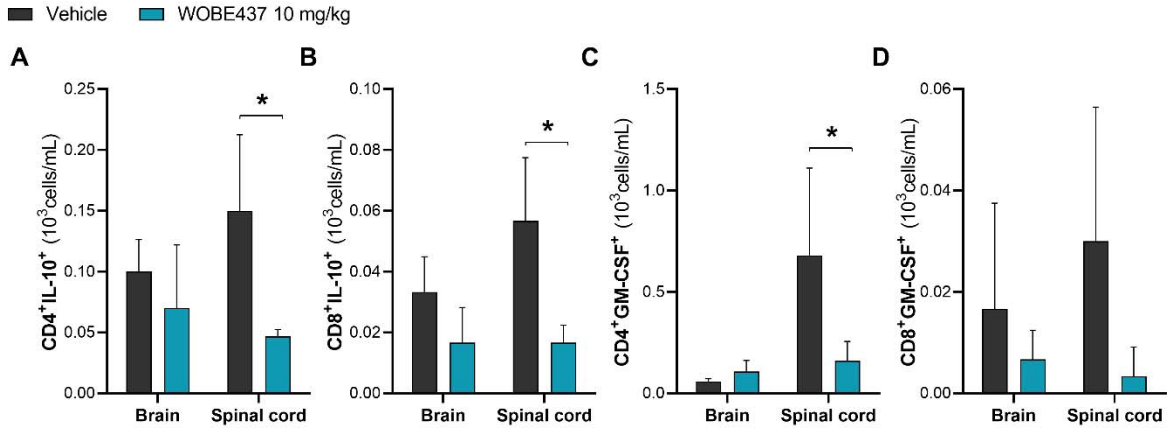
The day of onset is shown as the number of days post immunization. Only mice showing clinical symptoms were included in the study. Vehicle n= 37; WOBE437 (10 mg/kg) n= 29, Rimonabant (3 mg/kg) n= 17; WOBE437 + Rimonabant n= 20; AM630 (3 mg/kg) n= 16; WOBE437 + AM630 n= 21.

Figure S-2. Evaluation of body weight changes in groups treated in combination with CB1 and CB2 receptor antagonists.



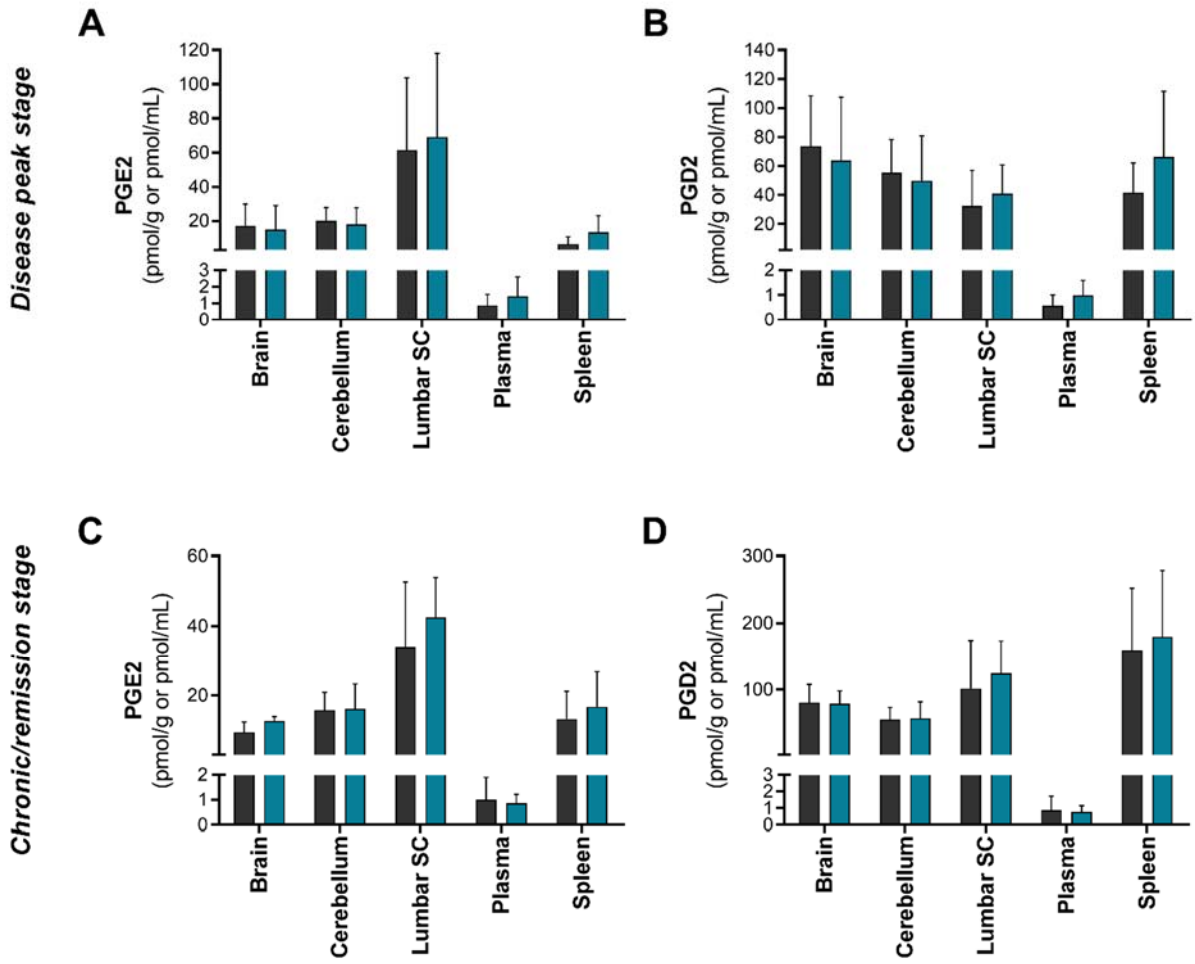
Time course of body weight changes from day -10 to day 20, onset is represented at day 1 (red line). Rimonabant (SR1, 3 mg/kg) was used as CB1 receptor antagonist / inverse agonist and AM630 (3 mg/kg) was used as CB2 receptor antagonist / inverse agonist. Administration of vehicle (dimethyl sulfoxide) and all compounds started at the individual day of onset (day 1) of each mouse, injections (20 µL) were done i.p. once per day during 20 days. Data show mean ± SEM; group size (mice per group): vehicle n= 37, WOBE437 n= 29, SR1 n= 17, SR1 + WOBE437 n= 20, AM630 n= 16, AM630 + WOBE437 n= 21. Data show the summary of two or four different cohorts, only mice showing symptoms were included in the study.

Figure S-3. Evaluation of IL-10 or GM-CSF producing $CD4^+$ and $CD8^+$ T cells isolated from brain and spinal cord of EAE female C57BL/6 mice treated with vehicle or WOBE437.



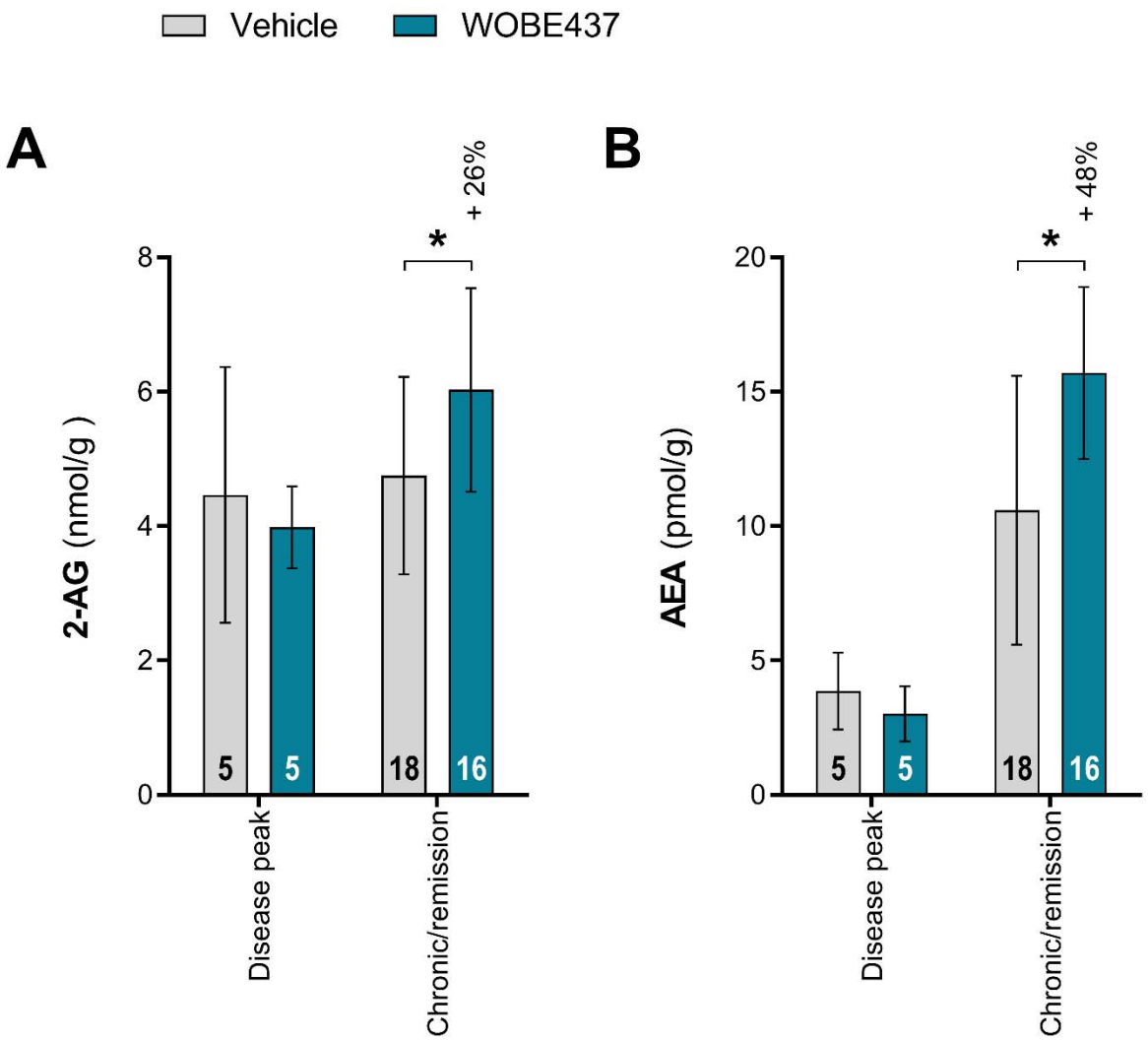
Lymphocytes were gated by $CD45^+$high expression, then gated in either (A) $CD4^+IL\text{-}10^+$ and (B) $CD8^+IL\text{-}10^+$ T cell subpopulations or (C) $CD4^+GM\text{-}CSF^+$ and (D) $CD8^+GM\text{-}CSF^+$ T cell subpopulations. Infiltrating immune cells were isolated from brain or spinal cord collected at the peak of disease (4-6 days post-treatment, day 1= onset). Daily treatment (i.p., 20 µL) with vehicle (DMSO) or WOBE437 (10 mg/kg) started at the onset of disease. Data show mean ± SD of infiltrating immune cells isolated from the brain or spinal cord of n = 6 mice per group. Data is depicted as a summary of three independent experiments including n = 2 mice (pooled) per group. Isolated cells were resuspended in 1 mL FACS buffer and cellular count was assessed using the volumetric sample and sheath fluid delivery system of the Attune NxT cytometer. Statistical differences were determined using Mann-Whitney test. *, $p < 0.05$ compare to vehicle group. IL-10, interleukin-10; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Figure S-4. Quantification of prostaglandin levels in central and peripheral tissues collected from EAE female C57BL/6 mice treated either with vehicle or WOBE437.



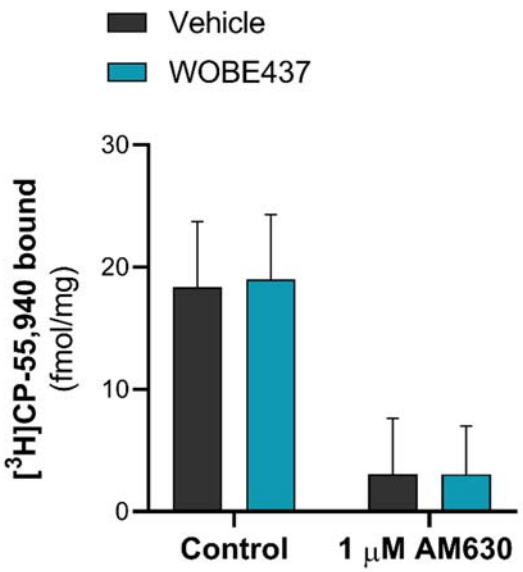
(A) Prostaglandin E2 (PGE2) and (B) Prostaglandin D2 (PGD2) were quantified in brain, cerebellum, lumbar spinal cord (lumbar SC), plasma and spleen at the peak of the disease (4-6 days after onset; average harvest day was 5 for both groups). (C) PGE2 and (D) PGD2 were quantified in brain, cerebellum, lumbar SC, plasma and spleen at the chronic/remission stage (20 days after onset). PGE2 and PGD2 were quantified using LC-MS/MS and values were normalized to the tissue amount (g) or volume (mL). Vehicle (DMSO) and WOBE437 were given i.p. (20 µL) starting at the disease onset (day 1). Sample size numbers (indicated as vehicle/WOBE437) were the following: (A, B) brain n= 8/9, cerebellum n= 8/9, Lumbar SC 5/6, plasma 8/9; (C) brain n= 21/17, cerebellum n= 20/15, Lumbar SC 13/7, plasma 21/17; (D) brain n= 21/17, cerebellum n= 20/15, Lumbar SC 13/7, plasma 22/22. Data show mean ± SD. Statistical differences were analyzed using Mann-Whitney test.

Figure S-5. Quantification of endocannabinoid levels in spleen samples collected from EAE female C57BL/6 mice treated either with vehicle or WOBE437.



(A) 2-arachidonoylglycerol (2-AG) and (B) anandamide (AEA) were quantified in spleen tissues harvested either at the peak of the disease (4-6 days after onset; average harvest day was 5 for both groups) or at the chronic/remission stage (20 days after onset). 2-AG and AEA were quantified using LC-MS/MS and values were normalized to the tissue amount (g). Vehicle (dimethyl sulfoxide) and WOBE437 were given i.p. (20 µL) starting at the disease onset (day 1), the corresponding sample size (n) are shown at the bottom of each bar. Data show mean ± SD. Statistical differences were determined using Mann-Whitney test. *, $p < 0.05$ compare to vehicle group; if significant, percentage of increase is reported above.

Figure S-6. Evaluation of CB2 receptor abundance in spleen membrane preparations from EAE female C57BL/6 mice treated either with vehicle or WOBE437.



Comparative CB2 receptor abundance was evaluated in spleen membrane preparations after incubation with 2 nM [^{3}H]CP-55940 and coincubation with vehicle (DMSO, control samples), 1 μM AM630 or 10 μM WIN-55212-2 to determine nonspecific binding. CB2 receptor binding was confirmed by coincubation with AM630, with 17.9% and 15.4% residual binding to CB1 receptors in the vehicle- and WOBE437-treated mice, respectively. Daily treatments (i.p., 20 μL) in the EAE mice started individually on the day of disease onset, spleen tissues were harvested after 20 days. n= 6 mice per group. Data show mean values ± SD. Statistical differences were determined using two-way ANOVA and Tukey's multiple comparison test.