Supplementary material

Supplemental Methods and Materials

Surgery (ovariectomy)

To eliminate the effects of circulating endogenous estrogens during the experiments, ovariectomy was performed in female mice when they were 4 to 5 weeks old. Mice were anesthetized by inhalational anesthesia with isoflurane. Carprofen (1.2 mg/ml per animal, Zoetis) and 0.5 ml of saline was injected subcutaneously before the procedure. The fur above the lateral dorsal back was removed and the skin was sterilized with betadine and alcohol scrubs. Bilateral incisions were made into the back. Hemostat was placed on the fallopian tubes, and the ovaries were removed. Muscle layer was closed with absorbable suture and the skin layer was closed with wound clips. Amoxicillin (50 mg/ml, Virbac) was added to the water for 5 days for antibiotics (0.5 mg/ml water) and another dose of carprofen was given within 24 h following surgery. Wound clips were removed 7 days later. All procedures were approved by the Office of Animal Research Oversight and UCLA institutional animal care and use committee, known locally as the Chancellor's Animal Research Committee.

Antibodies used for Immunofluorescence and CNS immune cell staining

Primary antibodies: anti-ERβ 1:100 (ab3577, Abcam), anti-ERβ 1:500 (68-4, EMD Millipore), anti-EGFP 1:1000 (NB100-1700, Novus Biologicals), anti-NF200 1:750 (N4142, Sigma), anti-SMI32 1:1000 (NE1023, EMD Millipore), anti-βAPP 1:200 (51-2700, Thermo Fisher Scientific), anti-MBP 1:750 (MAB386, EMD Millipore), anti-CNPase 1:500 (MAB326R, EMD Millipore), anti-CD3 1:2000 (1F4, BD Biosciences), anti-Iba1 1:500 (sc-32725, Santa Cruz Biotechnology), anti-Iba1 1:10,000 (Wako Chemicals), anti-MHCII (I-A/I-E) 1:400 (M5/114.15.2, Biolegend), anti-iNOS 1:500 (AB5384, EMD Millipore), anti-Arg1 1:100 (BD Biosciences), anti-Olig2 1:1000 (AB9610, EMD Millipore), anti-Olig2 1:200 (MABN50, EMD Millipore), anti-NG2 1:500 (AB5320 EMD Millipore), anti-CC1 1:500 (OP80, Calbiochem), and anti-GSTπ 1:1000 (ADI-MSA-102, Enzo Life Sciences).

Secondary antibodies: all secondary antibodies (1:1000) were produced from goat and the followings were used for staining the tissues: anti-rabbit-Cy3 (AP132C, EMD Millipore), anti-rabbit-DyLight®649 (AP187SD, EMD Millipore), anti-mouse-DyLight®649 (AP181SD, EMD Millipore), anti-rat-Cy3 (AP136C, EMD Millipore), and anti-rat-Cy5 (AB6565, Abcam).

Primer list for quantitative PCR

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- mouse Actβ (5′-GGCTCCTAGCACCATGAAGA-3′ and 5′-ACTCCTGCTTGCTGATCCAC-3′)
- mouse iNOS (5′-GTGACACACAGCGCTACAAC-3′ and 5′-GATGGTCACATTCTGCTTCTGG-3′)
- mouse T-bet (5′-ATGCCAGGGAACCGCTTAT-3′ and 5′-ATTGTTGGAAGCCCCCTTGT-3′)
- mouse IL-10 (5′-CAGTACAGCCGGGAAGACAA-3′ and 5′- TGGCAACCCAAGTAACCCTTA-3′)
- mouse CCR2 (5′-CATCTGCAAAAACAAATCAAAGGA-3′ and 5′-GACAAGGCTCACCATCATCG-3′)
- mouse Arginase (5′-ACATTGGCTTGCGAGACGTA-3′ and 5′-ATCGGCCTTTTCTTCCTTCCC-3′)
- mouse YM-1 (5′-GCAGAAGCTCTCCAGAAGCAAT-3′ and 5′-TCCCTTCTATTGGCCTGTCCT-3′)
- mouse ERβ (5′-ATTCTTCTCAAGCAGGTGGCCC-3′ and 5′-TTGGATCTGGTGCAGCAAGG-3′)

Reconstitution Rate

Mice were anesthetized by inhalational anesthesia with isoflurane and blood was drawn by retro-orbital puncture. Briefly, 100 ul of blood was collected using 0.5 M EDTA coated 50 µl microcapillary pipets (Kimble Glass). Collected blood was treated with anti-CD16/32 (93, BioLegend) for 10 min at RT for blocking, then cells were stained with premixed combination of antibodies: PE-anti-CD45.1 (A20, Biolegend) and FITC-anti-CD45.2 (104, Biolegend), for 30 min at RT. Stained cells were subjected to RBC lysis/fixation buffer (Biolegend) and washed thoroughly for flow cytometry. Flow cytometry was performed using the BD LSRFortessa cytometer at the UCLA Jonsson Comprehensive Cancer Center (JCCC) and Center for AIDS Research Flow Cytometry Core Facility.

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			P value	Degrees of freedom
Fig2.	Multiple comparison			
	WT-V vs WT-ERβ	***	0.0002	t(26)=4.975
	CKO-V vs CKO-ERβ	ns	> 0.9999	t(26)=1.053
	WT-V vs CKO-V	ns	0.2573	t(26)=2.129
	WT-V vs CKO-ERβ	ns	> 0.9999	t(26)=1.086
	WT-ERβ vs CKO-V	ns	0.0592	t(26)=2.784
	WT-ERβ vs CKO-ERβ	**	0.0039	t(26)=3.878
Fig5.	Multiple comparison			
	Olig1-WT-V vs Olig1-WT-ERβ	**	0.0095	t(17)=3.751
	Olig1-CKO-V vs Olig1-CKO-ERβ	ns	> 0.9999	t(17)=0.9283
	Olig1-WT-V vs Olig1-CKO-V	ns	> 0.9999	t(17)=0.01304
	Olig1-WT-V vs Olig1-CKO-ERβ	ns	> 0.9999	t(17)=1.004
	Olig1-WT-ERβ vs Olig1-CKO-V	*	0.0231	t(17)=3.343
	Olig1-WT-ERβ vs Olig1-CKO-ERβ	ns	0.208	t(17)=2.296
Fig6.	Multiple comparison			
	WT→WT-V vs WT→WT-ERβ	*	0.0188	t(25)=3.269
	WT→CKO-V vs WT→CKO-ERβ	**	0.0077	t(25)=3.629
	WT→WT-V vs WT→CKO-V	ns	> 0.9999	t(25)=0.06194
	WT→WT-V vs WT→CKO-ERβ	**	0.0067	t(25)=3.684
	WT→WT-ERβ vs WT→CKO-V	*	0.0214	t(25)=3.217
	WT→WT-ERβ vs WT→CKO-ERβ	ns	> 0.9999	t(25)=0.1953
Fig7.	Multiple comparison			
	WT→WT-V vs WT→WT-ERβ	***	0.0005	t(20)=4.932
	CKO→WT-V vs CKO→WT-ERβ	ns	> 0.9999	t(20)=1.169
	WT→WT-V vs CKO→WT-V	ns	> 0.9999	t(20)=0.4652
	WT→WT-V vs CKO→WT-ERβ	ns	0.7717	t(20)=1.585
	WT→WT-ERβ vs CKO→WT-V	**	0.0015	t(20)=4.435
	WT→WT-ERβ vs CKO→WT-ERβ	ns	0.0866	t(20)=2.679

Supplemental Table 1. Statistics of clinical EAE severity scores shown in Figures. Repeated measures two-way ANOVA, with Bonferroni's multiple comparisons test. Shading highlights ERβ ligand (ERβ) versus vehicle control (V) treatment in WT, as well as ERβ ligand (ERβ) versus Vehicle control (V) treatment in each conditional knock out (CKO).

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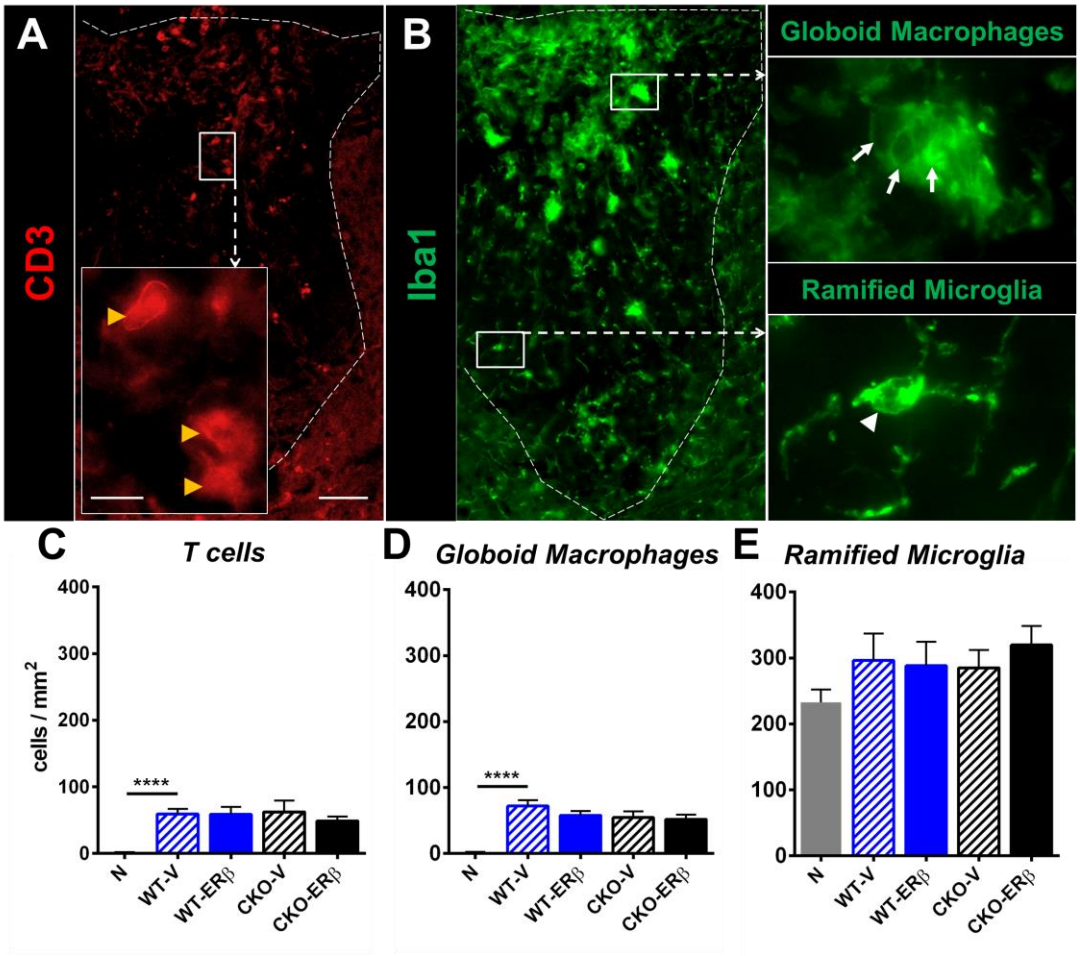
		Mean Clinical Scores						Mean Difference	Standard Error	P value
Fig 2	**Ex. 1**	WT-V	n=9	3.389	WT-ERβ	n=6	2.569	0.8194	0.1647	0.0002
		CKO-V	n=7	3.054	CKO-ERβ	n=8	3.224	-0.1704	0.1617	> 0.9999
	Ex. 2	WT-V	n=9	2.963	WT-ERβ	n=9	2.191	0.7716	0.3604	0.0422
		CKO-V	n=4	2.822	CKO-ERβ	n=7	3.431	0.3829	0.4792	0.4317
	Ex. 3	WT-V	n=8	3.840	WT-ERβ	n=7	3.063	0.7768	0.2281	0.0023
		CKO-V	n=7	3.690	CKO-ERβ	n=6	3.796	-0.1058	0.2452	0.6699
Fig 5	**Ex. 1**	Olig1-WT-V	n=6	3.383	Olig1-WT-ERβ	n=7	2.350	1.033	0.2754	0.0095
		Olig1-CKO-V	n=4	3.388	Olig1-CKO-ERβ	n=4	3.063	0.3250	0.3501	> 0.9999
	Ex. 2	Olig1-WT-V	n=5	2.943	Olig1-WT-ERβ	n=7	1.684	1.259	0.5481	0.0320
		Olig1-CKO-V	n=6	3.190	Olig1-CKO-ERβ	n=7	2.724	0.4660	0.5208	0.3811
	Ex. 3	Olig1-WT-V	n=8	2.361	Olig1-WT-ERβ	n=8	1.753	0.6076	0.2926	0.0471
		Olig1-CKO-V	n=8	2.316	Olig1-CKO-ERβ	n=8	2.420	-0.1042	0.2926	0.7245
Fig 6	**Ex. 1**	WT→WT-V	n=9	3.315	WT→WT-ERβ	n=5	2.158	1.156	0.3537	0.0188
		WT→CKO-V	n=9	3.296	WT→CKO-ERβ	n=6	2.083	1.213	0.3343	0.0077
	Ex. 2	WT→WT-V	n=5	3.042	WT→WT-ERβ	n=5	1.993	1.049	0.4848	0.0498
		WT→CKO-V	n=5	3.039	WT→CKO-ERβ	n=4	2.424	0.6153	0.4599	0.2039
Fig 7	**Ex. 1**	WT→WT-V	n=6	3.579	WT→WT-ERβ	n=8	2.615	0.9641	0.1955	0.0005
		CKO→WT-V	n=6	3.481	CKO→WT-ERβ	n=4	3.208	0.2731	0.2336	> 0.9999

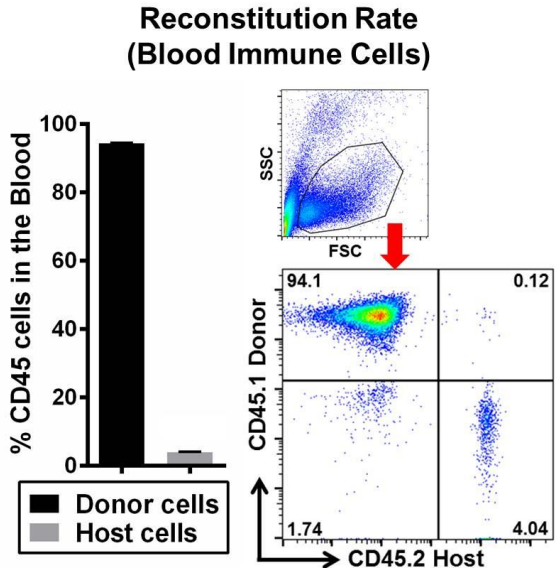
Supplemental Table 2. Mean EAE clinical scores, sample sizes, standard errors and significance levels for each comparison in all EAE experiments. Repeated measures two-way ANOVA, with Bonferroni's multiple comparisons test. Wildtype vehicle control (WT-V) or ERβ ligand (WT-ERβ) treatment and conditional knockout vehicle control (CKO-V) or ERβ ligand (CKO-ERβ) treatment.

Supplementary material



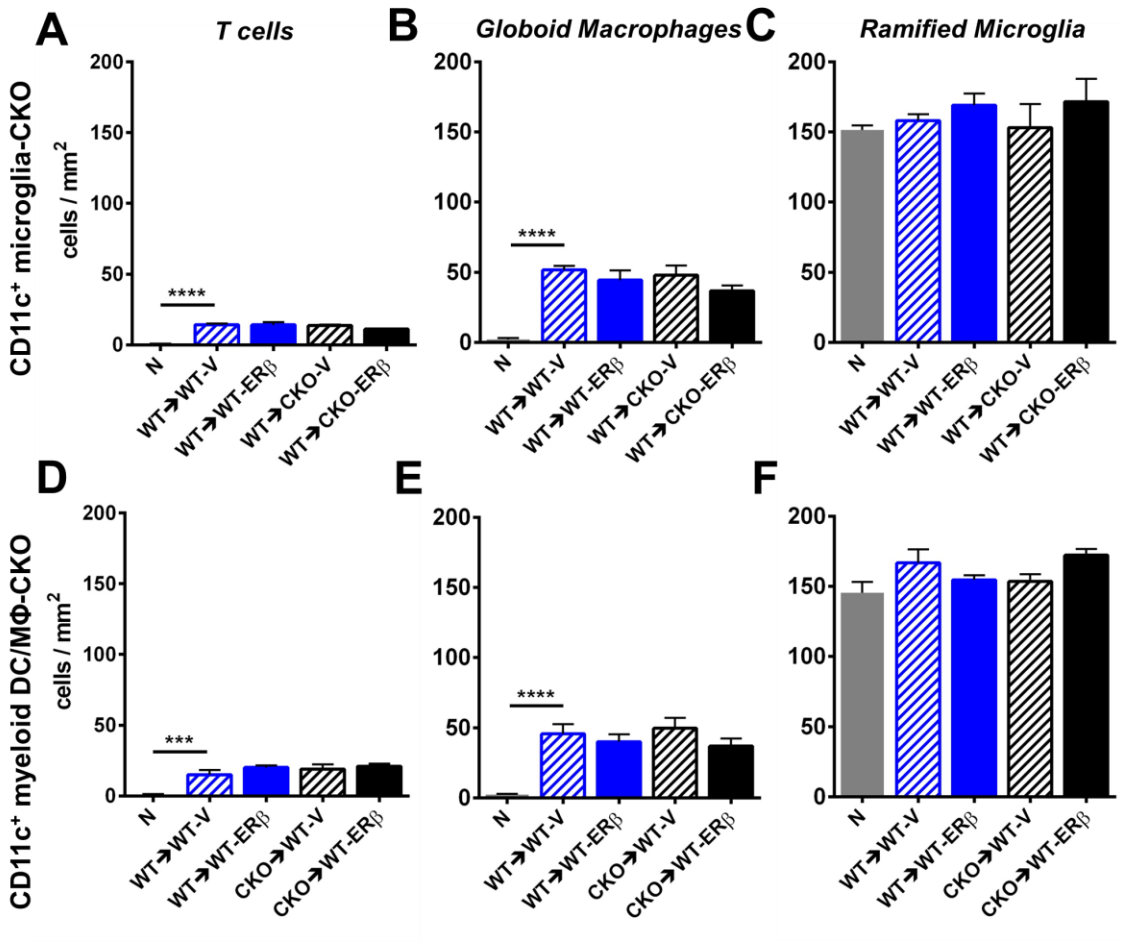
Supplemental Figure 1. No quantitative effects on CNS resident and infiltrated immune cells. (A and B) Immunofluorescence images of spinal cord tissues stained with CD3 (red) and Iba1 (green). Dotted lines represent the area of dorsal white matter in the spinal cord. **(A)** CD3 staining of T cells in CNS (orange arrowhead) and **(B)** Iba1 staining of globoid macrophages (white arrow) and ramified microglia (white arrowhead). Scale bar = 50 μm and 5 μm (inset). Inset boxes are 40x images. Quantitative analysis of **(C)** infiltrated $CD3^+$ T cell, **(D)** $Iba1^+$ globoid macrophages in spinal cord white matter show that all EAE groups were increased compared to healthy controls, but not different from each other. **(E)** $Iba1^+$ ramified microglia cell numbers were no different between groups. ****$P < 0.0001$.

Supplementary material



Supplemental Figure 2. Reconstitution efficiency in BMC mice. Blood immune cells were stained with PE-CD45.1 and FITC-CD45.2 antibodies for flow cytometry analysis. Reconstitution rates were calculated based on the percentage donor vs host $CD45.1^+$ or $CD45.2^+$ cells among total $CD45^+$ blood immune cells. Shown is a representative experiment with reconstitution efficiency of 94.28 ± 0.23%.

Supplementary material



Supplemental Figure 3. No quantitative effects on CNS resident and infiltrated immune cells in bone marrow chimeric EAE mice. Quantitative analysis of **(A and D)** infiltrated $CD3^+$ T cells and **(B and E)** $Iba1^+$ globoid macrophages in the spinal cord white matter of $CD11c^+$ microglia CKO and $CD11c^+$ myeloid DC/MΦ CKO EAE mice, respectively, showed that all EAE groups were increased compared to healthy controls, but no different from each other. **(C and F)** CNS resident $Iba1^+$ ramified microglia cell numbers in the spinal cord white matter were no different between groups. ***P < 0.001; ****P < 0.0001.