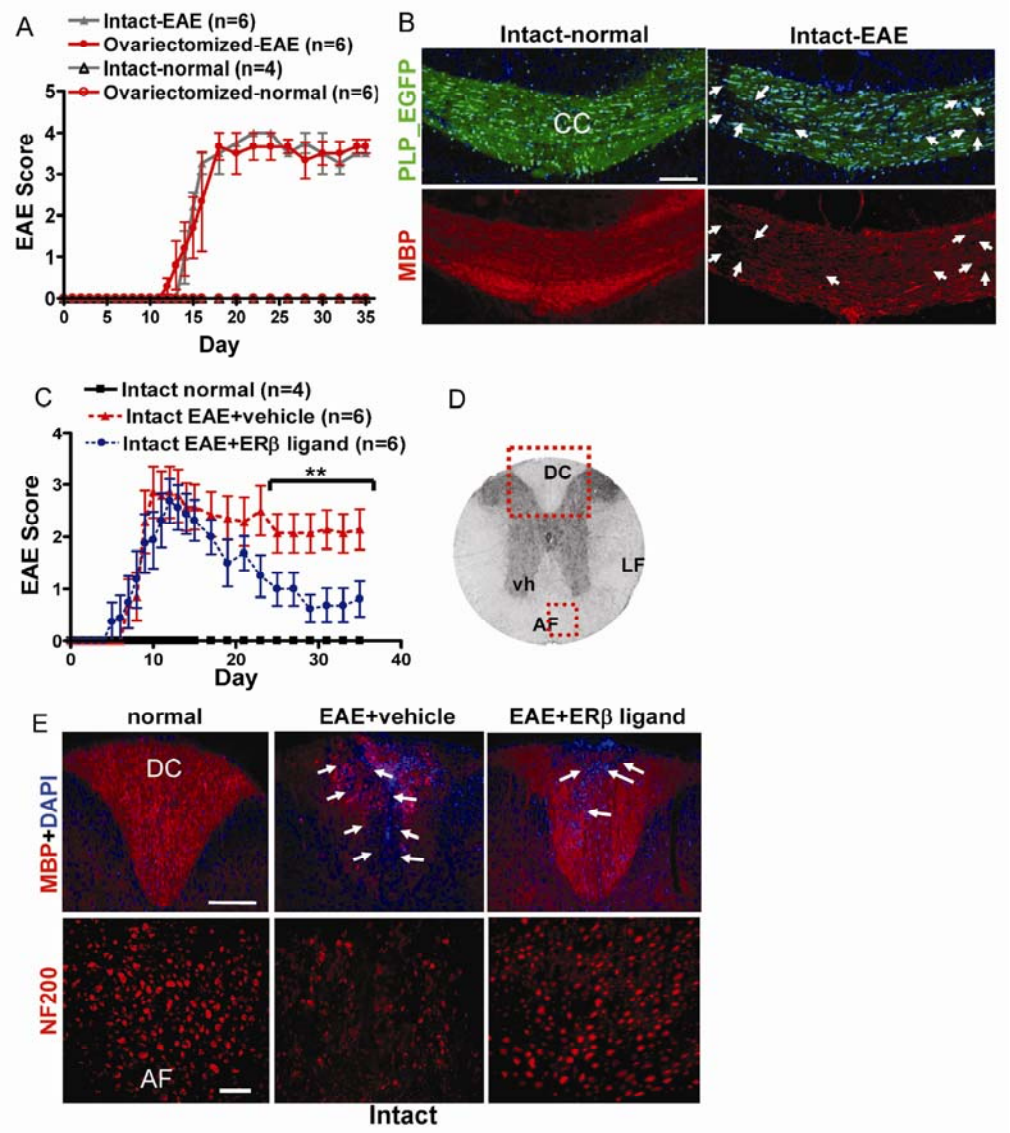
Supplementary Figures

Supplementary Figure 1. Similar EAE clinical scores and histopathology in ERβ ligand treated EAE intact and ovariectomized mice



(A) Active EAE was induced with MOG peptide in age matched intact and ovariectomized PLP_EGFP C57BL/6 female mice and scored using the standard EAE grading scale. There was no significant difference in early or late disease. Normal intact and ovariectomized mice did not show any disease and their clinical scores remained zero through out the experiment. Number of mice in each group were intact normal, n=4; intact EAE, n=6; ovariectomized normal, n=6, gonadectomized EAE, n = 6.

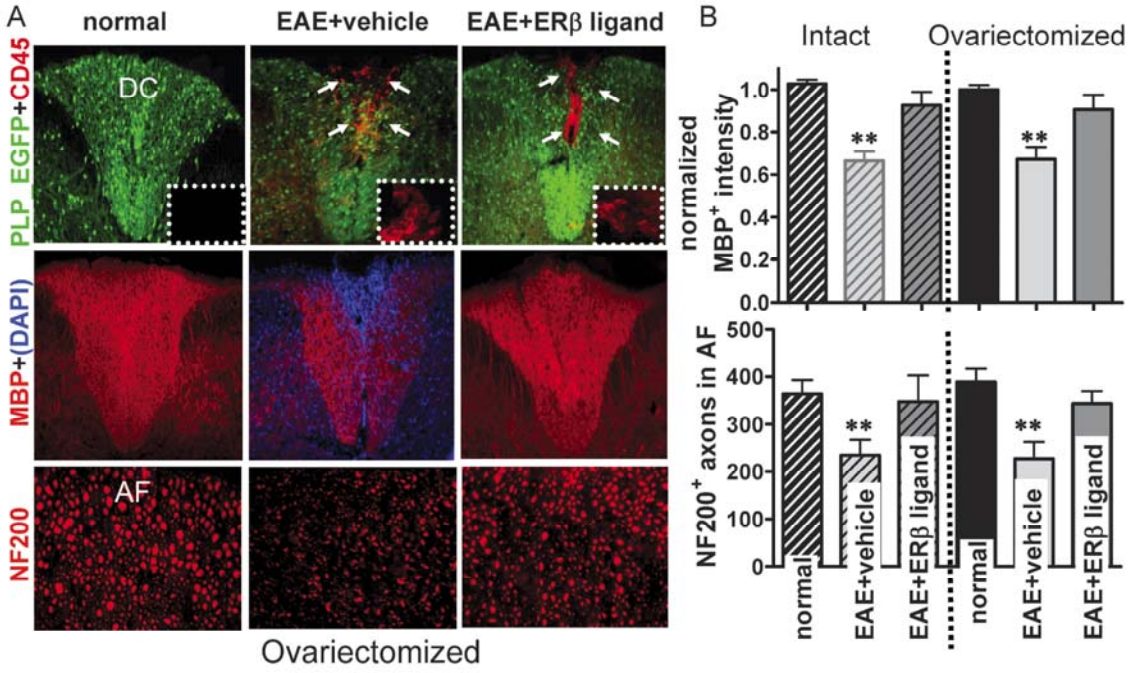
(B) Representative PLP_EGFP expressing (green), MBP (red) and DAPI nuclei (blue) stained brain callosal sections (10X magnification) from intact normal and intact EAE mice were all sacrificed at day 36 (late) post-disease induction. Compared to intact normal mice, the CC of EAE mice had an increase in the total number of infiltrating cells (represented by $DAPI^+$ cells). This was accompanied by a reduction in PLP_$EGFP^+$ cells, as well as PLP_EGFP white matter and MBP immunostaining intensity (white arrows). Scale bar is 100 μm.

(C) In a separate experiment intact PLP_EGFP C57BL/6 female mice were given subcutaneous injections of ERβ ligand during active EAE, and scored using the standard EAE grading scale. Similar to ERβ ligand-treated gonadectomized mice, ERβ ligand-treated intact mice, were not significantly different as compared to vehicle treated mice early in disease (up to day 20 after disease induction), but then became significantly improved later during EAE, (starting at day 22-25 after disease induction, $p<0.001$, ANOVA Friedman test). Normal intact mice did not show any disease and their clinical scores remained zero through out the experiment. Number of mice in each group were normal, n=4; EAE+vehicle, n=6; EAE+ERβ ligand, n=6.

(D) Hemotoxylin-Eosin stained thoracic spinal cord section from ovariectomized normal mice anatomically labeled to identify dorsal column (DC), anterior funiculus (AF), lateral funiculus (LF) and ventral horn. The areas used for quantification are marked around DC and in AF region with dashed red lines.

(E) Representative dorsal column (10X magnification) immunostained with MBP (red) and DAPI nuclei (blue) in the upper panel and anterior funiculus (AF) immunostained with NF200 (red-40X magnification) in the lower panel are from intact normal, EAE+vehicle and EAE+ERβ ligand mice all sacrificed at day 36 (late) post-disease induction. Compared to intact normal mice, the spinal cord of EAE+vehicle mice had an increase in the total number of infiltrating cells (represented by $DAPI^+$ cells) after induction of EAE. This was accompanied by a reduction in MBP immunostaining intensity (white arrows) and NF200+ axon numbers. Scale bars are 100 μm and 10μm respectively.

Supplementary Figure 2. EAE induced spinal cord inflammation, demyelination and axon degeneration is similar in intact and ovariectomized mice.



(A) Shown here are representative thoracic spinal cord brain sections from age-matched ovariectomized (normal, day 36 EAE+vehicle, and day 36 EAE+ERβ ligand) animal. Infiltrating $CD45^+$ microglia (red) are imaged at 10X and dashed box inset at 40X magnification are seen in EAE and EAE+ERβ ligand treated dorsal column. Second panel shows MBP+ (red) immunostaining at 10X in the dorsal column (only the EAE+vehicle section shows $DAPI^+$ nuclei) and the third panel shows $NF200^+$ (red) axons imaged at 40X. Compared to intact normal mice, the dorsal column of EAE mice and EAE+ERβ ligand-treated had increased infiltrating $CD45^+$ cells. Similar to previously observed, MBP fluorescence intensity and axon numbers in EAE+ERβ ligand treated groups were significantly higher than vehicle-treated EAE.

(B) Myelin immunostaining intensity and axon numbers were quantified. Both intact and ovariectomized EAE groups that were treated with ERβ ligand showed significant increase in myelin density and axon numbers as compared to EAE+vehicle groups (*$p<0.05$; **$p<0.001$, ANOVAs; Bonferroni's multiple comparison post-test; n=4).