SUPPLEMENTAL MATERIAL

Han et al., http://www.jem.org/cgi/content/full/jem.20101974/DC1

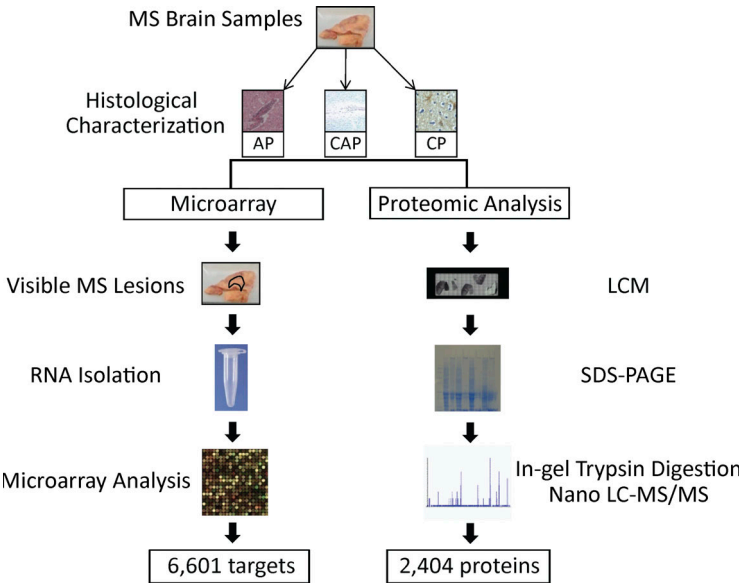

Figure S1. Flow chart of microarray and proteomic analysis of MS lesions. MS lesions were characterized histologically into AP, CAP, and CP. RNA and protein samples were extracted from the same lesions and analyzed by microarray and mass spectrometry (Han et al., 2008), respectively. The total number of targets identified by each analysis is depicted. LCM, laser capture microscopy; Nano LC-MS/MS, nano–liquid chromatography tandem mass spectrometry.

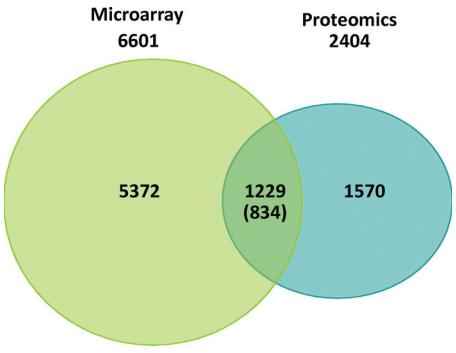

Figure S2. Intersection of targets identified by microarray and proteomic analysis. Tissue containing AP, CAP, and CP were analyzed by microarray analysis and by mass spectrometry. Microarray analysis identified 6,601 RNA targets, whereas the corresponding proteomic experiment identified 2,404 protein targets. Only 1,229 RNA targets (of the 6,601 total, ~20% of identified) mapped to 834 proteins identified in the proteomic study (~30% of all proteins identified). The majority of the targets (5,372 RNA targets and 1,570 proteins) had no overlap between the two platforms.

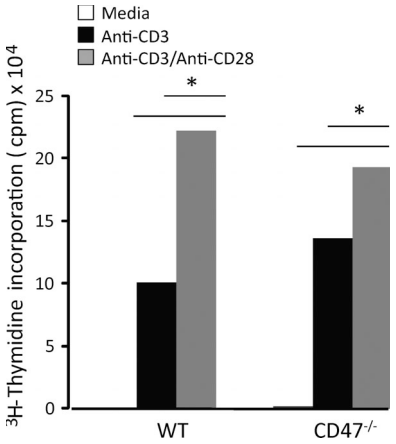

Figure S3. CD47$^{-/-}$ T cells proliferate when activated with anti-CD3 or anti-CD28 in vitro. CD3-positive T lymphocytes from WT naive C57BL/6 or CD47$^{-/-}$ mice isolated by negative affinity selection using anti-CD3 affinity purification (BD). WT or CD47$^{-/-}$ CD3$^+$ T cells were incubated in 96-well round-bottom plates (10^6 cells per well), activated with plate-bound anti-CD3 alone or in combination with 5 µg/ml anti-CD28, pulsed with [^{3}H]thymidine at either 24, 48, or 72 h, and harvested 16 h later. Thymidine incorporation was measured using a β scintillation counter. *, $P < 0.05$ by Student's *t* test.

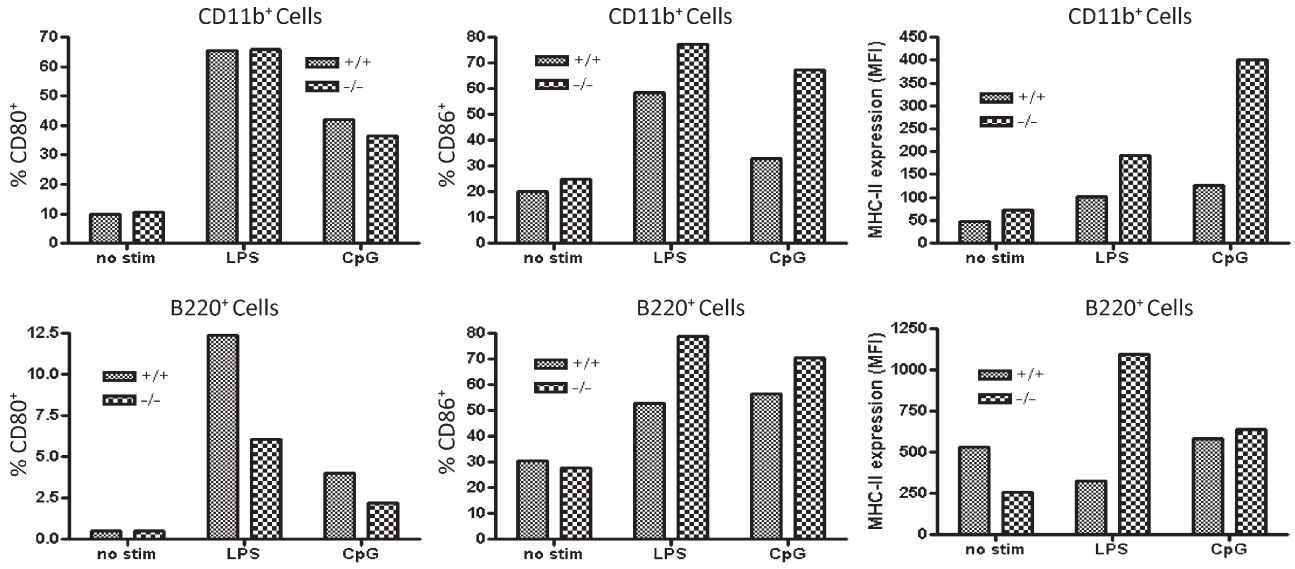

Figure S4. CD47$^{-/-}$ APCs express co-stimulatory molecules upon either LPS or CpG activation. Splenocytes from WT or CD47$^{-/-}$ presymptomatic EAE mice (days 7–10 after immunization) were cultured with either 100 ng/ml LPS or CpG for 48–72 h and then analyzed by flow cytometry for expression of activation markers (CD80, CD86, and MHCII) in APCs. MFI, mean fluorescent intensity.

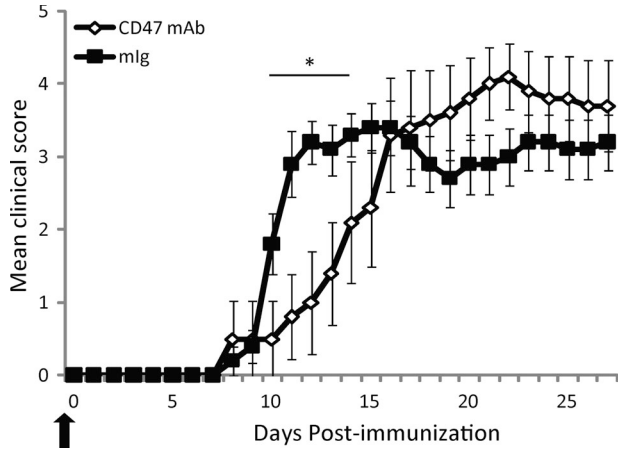

Figure S5. mAb CD47 treatment lessens EAE severity. Mean clinical scores ± SEM of C57BL/6 WT EAE mice treated with 200 µg CD47 mAb (alternate day i.p. injections [open diamonds] or isotype control IgG [mIg; closed squares] at similar doses). *, $P < 0.05$ by Mann-Whitney analysis. The arrow indicates onset of treatment with either mAb CD47 or control Ig (day 0). This experiment was performed twice with $n = 10$ mice per arm.

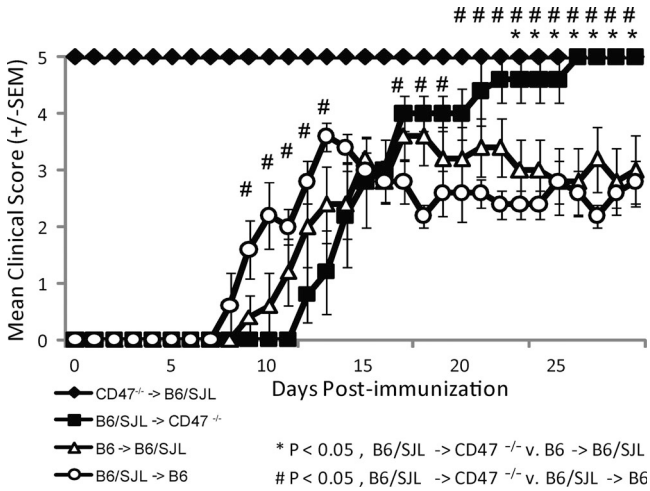

Figure S6. Bone marrow chimera experiment. 5-wk-old WT C57BL/6J, CD47$^{-/-}$ on C57BL/6J background, or B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ hosts were irradiated (total 1,100 cGy in two divided doses) from a 200-Kv x-ray source and injected with donor cells i.v. within 24 h. Bone marrow was harvested from the femur of WT C57BL/6, CD47$^{-/-}$, or B6.SJL-*Ptprc*^{a} *Pepc*^{b}/BoyJ mice and passaged through a 70-µM filter to make a single-cell suspension. All host mice received 2×10^6 bone marrow cells for reconstitution. Host mice were kept on antibiotic water (25 µg/ml neomycin/0.3 U/ml polymyxin B; Sigma-Aldrich) for the first 28 d. After reconstitution, EAE was induced in these mice, and mice were followed clinically daily (see EAE, treatment with mAb CD47, immune cell proliferation, and cytokine analysis). This experiment was performed once with $n = 5$ mice per arm. Standard error was calculated from clinical scores from these EAE mice.

Table S1, included as an Excel file, shows the MS brain lesion transcriptome.
Table S2, included as an Excel file, shows the MS brain lesion proteome.
Table S3, included as an Excel file, compares the MS brain lesion transcriptome and proteome.

REFERENCE

Han, M.H., S.I. Hwang, D.B. Roy, D.H. Lundgren, J.V. Price, S.S. Ousman, G.H. Fernald, B. Gerlitz, W.H. Robinson, S.E. Baranzini, et al. 2008. Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets. *Nature*. 451:1076–1081. http://dx.doi.org/10.1038/nature06559