ONLINE METHODS

Mice. Female SJL/J mice were purchased from Harlan Laboratories (Indianapolis, IN). Female BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). TCR transgenic mice expressing a TCR specific for $PLP_{139–151}$ (5B6) on the SJL/J background were the kind gift of V. Kuchroo (Harvard) and were bred in-house at Northwestern University. *Marco*$^{-/-}$ mice on the BALB/c background were kindly provided by L. Kobzik (Harvard). All mice were housed under specific pathogen–free conditions in the Northwestern University Center for Comparative Medicine and maintained according to protocols approved by the Northwestern University Institutional Animal Care and Use Committee.

Peptides. $PLP_{139–151}$ (HSLGKWLGHPDKF), $MOG_{35–55}$ (MEVGWYRSPF SRVVHLYRNGK) and $OVA_{323–339}$ (ISQAVHAAHAEINEAGR) were purchased from Genemed Synthesis. $PLP_{178–191}$ (NTWTTCQSIAFPSK) was purchased from Peptides International.

Induction and clinical evaluation of peptide-induced EAE. Peptide-induced and adoptive-transfer EAE was induced in SJL/J mice as previously reported[48,49]. Individual mice were observed daily, and clinical scores were assessed in a blinded fashion on a 0–5 scale as follows: 0, no abnormality; 1, limp tail or hindlimb weakness; 2, limp tail and hindlimb weakness; 3, hindlimb paralysis; 4, hindlimb paralysis and forelimb weakness; and 5, moribund. The data are reported as the mean daily clinical score. Paralyzed mice were given easier access to food and water.

Tolerance induction with antigen-coupled cells and microparticles. Tolerance was induced by i.v. injection of chemically treated Ag-SP, as described previously[11,18]. Briefly, spleens were removed from naive female mice, and the red blood cells were lysed. The splenocytes were incubated with ECDI (150 mg per 3.2×10^8 cells; Calbiochem) and peptide (1 mg/ml) on ice, shaking for 1 h. The coupled cells were washed three times and filtered through a 70-μM cell strainer to remove cell clumps. The Ag-SP were resuspended at 250×10^6 cells/ml in PBS. Each mouse received 50×10^6 Ag-SP in 200 μl of PBS given by i.v. injection at the indicated times before disease induction. This dosage represents delivery of a total of 15–20 μg of cell-bound peptide per mouse. Carboxylated PSB of various diameters were purchased from Polysciences (Warrington, PA). Peptide antigens were attached to particles using ECDI according to manufacturer's instructions (12.5 mg of polystyrene microparticles and 500 μg of peptide in the presence of 10 mg/ml ECDI). Five-hundred–nanometer carboxylated PLG microparticles were purchased from Phosphorex, Inc. (Fall River, MA), and peptide antigens were attached using ECDI as for the PSB. Mice received intravenous injections of approximately 9×10^9 microparticles comprising 15–20 μg of peptide, depending on the sequence used in the coupling reaction.

Antibodies and flow cytometry. Cells were isolated from the spleen or CNS as previously described[49]. Briefly, FcR blocking with CD16/32 (clone 2.4G2) was performed, followed by staining with various combinations of antibodies to the following: CD69 (clone H1.2F3)-allophycocyanin (APC), CD69-FITC, CD62L (clone MEL14)-APC/AlexaFluor750, CD44 (clone IM7)-phycoerythrin (PE)/Cy7, Foxp3 (clone FJK-16s)-APC, Foxp3-PE/Cy7, CD152 (clone UC10-4B9)-PE, PD-L1 (clone MIH5)–PE, IFN-γ (clone XMG1.2)–PE/Cy7, IL-17 (clone eBio17B7)–APC, CD90.1 (clone HIS51)-Pacific Blue and CD45 (clone 30-F11)-PE, all of which were purchased from eBioscience. CD25 (clone PC61)-FITC and CD25-APC, CD3 (clone 145.2C11)-APC-Alexa750 and CD4 (clone GK1.5)-PerCP were purchased from Becton-Dickinson. Cytometric data were collected on a FACS Canto flow cytometer (Becton-Dickinson). DiVa software was used for data acquisition and analysis (Becton-Dickinson).

DTH and *in vitro* proliferation assays. DTH was performed by a 24 h ear swelling assay as previously reported[11]. Prechallenge ear thickness was determined using a Mitutoyo model 7326 engineer's micrometer (Schlesinger's Tools, Brooklyn, New York). Immediately after, DTH responses were elicited by injecting 10 μg of peptide in 10 μl of PBS into the dorsal surface of the ear using a 100-μl Hamilton syringe fitted with a 30 gauge needle. The increase in ear thickness over the prechallenge measurements was determined 24 h after ear challenge. Results are expressed in units of 10^{-4} inches ± s.e.m. For proliferation assays, draining lymph nodes (axillary, brachial and inguinal) and/or spleens were harvested from naive mice or primed mice at the indicated times after disease induction, counted and cultured in 96-well microtiter plates at a density of 5×10^5 cells per well in a total volume of 200 μl of HL-1 medium (BioWhittaker; 1% penicillin and streptavidin and 1% glutamine). Cells were cultured at 37 °C with medium alone or with different concentrations of peptide antigen for 72h. During the last 24 h, cultures were pulsed with 1 μCi per well [^{3}H]TdR, and uptake was detected using a Topcount microplate scintillation counter. Results are expressed as the mean of triplicate cultures. IFN-γ and IL-17 concentrations were determined by Liqui-Chip analysis.

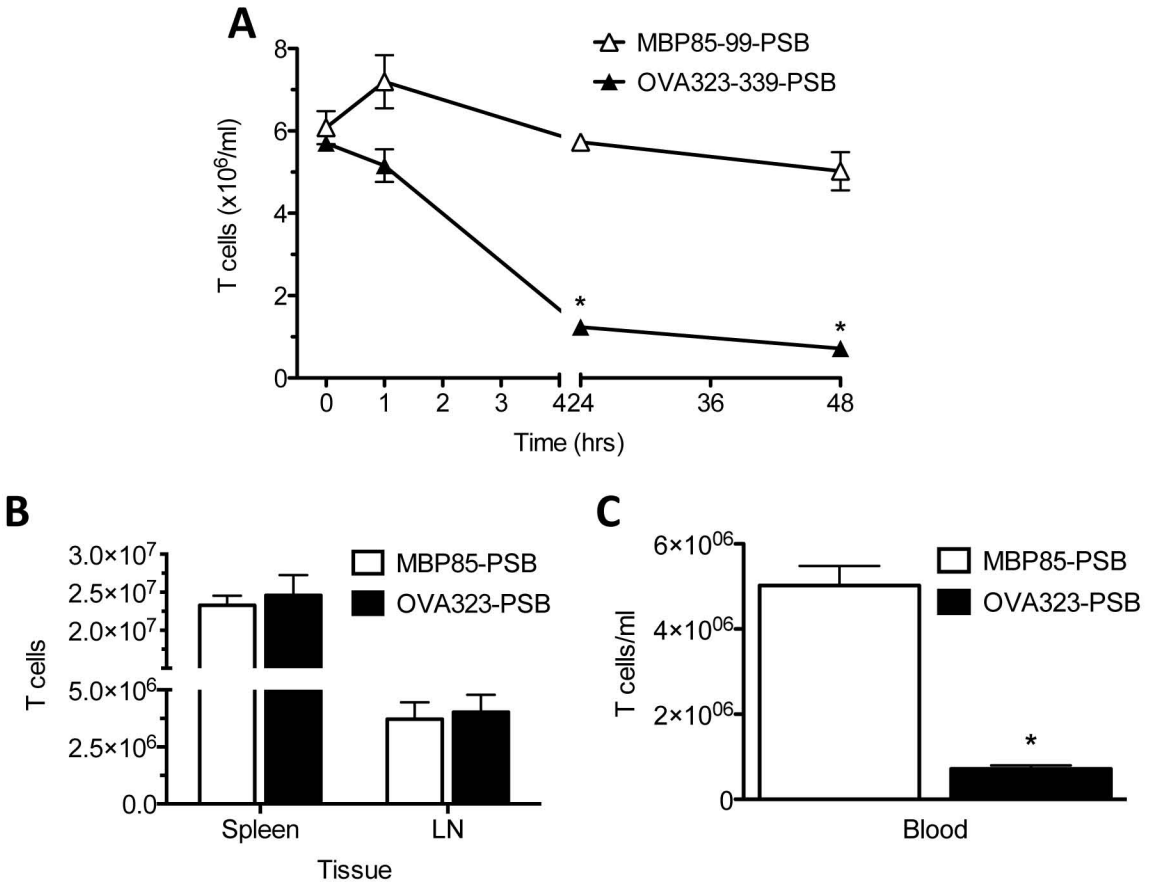
T_{reg} cell inactivation. T_{reg} cell inactivation was performed by injection of 500 μg per mouse anti-CD25 (clone PC61) as described previously[25].

T cell transfer assays. Naive T cells were isolated from the lymph nodes of healthy 5B6 mice by magnetic separation. Single-cell preparations of nodes were prepared, FcR blocked with 2.4G2 and labeled with $CD4^+$ T cell isolation reagents (Miltenyi Biotec). T cells were isolated using an AutoMACS magnetic separator (Miltenyi Biotec). Ninety-four to ninety-eight percent purity was routinely achieved. After isolation, 20×10^6 T cells per ml were fluorescently labeled in a 4 μM solution of carboxyfluorescein diacetate in PBS for 8 min at room temperature. The reaction was quenched by addition of a half volume of heat-inactivated FBS and an additional 5 min incubation at room temperature. Cells were washed twice in PBS before injection into the lateral tail vein of recipient mice (5×10^6 T cells per recipient). Forty-eight hours after transfer, recipient mice were treated with a variety of antigen-coupled microparticles or with antigen in CFA. At various times after treatment, spleens and lymph nodes were isolated, and transgenic T cells (identifiable by CD90.1 and transgene expression) in these organs were analyzed for cell division and a variety of surface and intracellular markers as described above.

Immunohistochemistry. Mice were infused with PBS or FITC-labeled $MOG_{35–55}$-PSB. Eighteen hours after infusion, spleens were dissected, fixed in paraformaldehyde for 30 min to 3 h at 4 °C in the dark and snap frozen in optimal cutting temperature compound. The blocks were stored at −80 °C in plastic bags to prevent dehydration. Six-micrometer–thick cross-sections were cut on a Reichert-Jung Cryocut CM1850 cryotome (Leica), mounted on Superfrost Plus electrostatically charged slides (Fisher), air dried and stored at −80 °C. Slides were stained using the Tyramide Signal Amplification Direct kit (NEN) according to the manufacturer's instructions. Nonspecific staining was blocked using either Ab that recognizes both CD16 and CD32 (FcIII/IIR, 2.4G2; BD Pharmingen) or 10% horse serum, as well as an avidin and biotin blocking kit (Vector Laboratories) in addition to the blocking reagent provided by the Tyramide Signal Amplification kit (NEN). Sections were then stained with primary [MARCO (BAF2956), SIGN-R1 (BAF1836) or SIGLEC-1 (BAF5610)] and secondary antibodies, as well as DAPI as previously described[12]. Sections were coverslipped with Vectashield mounting medium with DAPI (Vector Laboratories), and images were acquired using a Lica DM5000B fluorescent microscope and Advanced SPOT software. At least eight serial sections from each sample per group were analyzed at × 20, × 40 and × 100 magnification.

Statistical analyses. Comparisons of DTH responses, mean clinical disease scores, proliferation, cytokine responses or mean fluorescence intensities between any two groups of mice were analyzed by a standard two-tailed *t* test or one-way ANOVA, depending on the precise comparisons made. $P < 0.05$ was considered significant.

48. Bailey, S.L., Schreiner, B., McMahon, E.J. & Miller, S.D. CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4(+) T(H)-17 cells in relapsing EAE. *Nat. Immunol.* **8**, 172–180 (2007).
49. Schreiner, B., Bailey, S.L., Shin, T., Chen, L. & Miller, S.D. PD-1 ligands expressed on myeloid-derived APC in the CNS regulate T-cell responses in EAE. *Eur. J. Immunol.* **38**, 2706–2717 (2008).



Supplementary Figure 1. Effect of Ag-PSB on Antigen-Specific T Cells. Female DO11.10 mice were given i.v. treatments of 500nm carboxylated PSBs coupled to cognate antigen (OVA323-339) or irrelevant antigen (MBP85-99). **(A)** Peripheral blood was analyzed for T cell content at 1, 24 and 48h post-treatment. **(B&C)** At 48h, spleens, lymph nodes and peripheral blood from select mice were collected and OVA323-339-specific T cell content was quantified by flow cytometry using the DO11.10 TCR-specific antibody KJ-126. The total numbers of $CD4^+KJ\text{-}126^+$ cells (**B**) and the numbers of $CD4^+KJ\text{-}126^+$ cells/ml of peripheral blood (**C**) are shown. Results are representative of 3 separate experiments. *Numbers of $KJ\text{-}126^+$ T cells in the peripheral blood of OVA323-PSB treated mice is significantly less than those in the MBP85-99-PSB ($p < 0.001$, Student's t-test). Error bars indicate standard error of the mean.