Supporting Information

Hawkes and McLaurin 10.1073/pnas.0805453106

SI Methods

Materials. Phosphatidylcholine, cholesterol, *p*-amino-phenyl-α-D-mannopyranoside, dicholoromethylenediphosphonic acid disodium salt (clodronate), chitin (from crab shell), and thioflavin S were purchased from Sigma-Aldrich. Anti-CD163 and anti-CD206 antibodies were obtained from AbDSerotec, anti-human $A\beta_{40}$ and anti-human $A\beta_{42}$ antibodies were from Chemicon, anti-GAPDH was from Meridian Life Science, anti-Iba1 was purchased from Wako Pure Chemicals, and anti-GFAP antibodies, horseradish peroxidase-conjugated secondary antibodies, and the enhanced chemiluminescence kit were purchased from Dako. Fluorescently conjugated secondary antibodies and polyacrylamide electrophoresis gels (4%–20%) were purchased from Invitrogen. Human $A\beta_{40}$ and $A\beta_{42}$ ELISA kits were obtained from BioSource.

Depletion of Perivascular Macrophages. Multilaminar mannosylated liposomes were prepared by dissolving 178 mg of phosphosphatidylcholine and 27 mg of cholesterol in 8 mL of chloroform, to which 9.25 mg of *p*-amino-phenyl-α-D-mannopyranoside in 5 mL methanol was added in a round-bottomed flask. After evaporation, the lipid film was dispersed in 10 mL of PBS solution (0.1 M, pH 7.4) alone or containing 2.5 g of clodronate. Both preparations were kept under a nitrogen stream for 2 h at room temperature, sonicated for 3 min, washed, and resuspended in 4 mL of sterile PBS solution.

Cortical Blood Vessel Isolation. The brains of PBS-perfused mice were rapidly removed and dissected for cortices, which were homogenized in 1.5 mL of blood vessel isolation buffer (0.1M NH_4CO_3, 5 mM EDTA, 0.01% sodium azide and protease inhibitor mixture) by using a Dounce homogenizer (6 strokes). Homogenates were centrifuged (100,000 × *g*, 1 h, 4 °C) and pellets resuspended in 500 μL of 0.1M NH_4CO_3 plus 7% SDS (plus protease inhibitor mixture) and stirred for ≈4 h. Tissues were then sequentially filtered through 100-μm and 40-μm mesh filters to isolate blood vessel tufts from cortical filtrate.

Double Labeling and Fluorescent Immunocytochemistry. For CD163 plus GLUT-1/CD31, free-floating brain tissue sections were washed in PBS solution, blocked for 15 min with 15% normal goat serum, and incubated overnight with anti-CD163 (1:100). Sections were incubated with Alex Fluor 488-conjugated anti-mouse (1:200) for 2 h, rinsed in PBS solution and then processed for GLUT-1 (1:250) or CD31 (1:100) staining. Sections were rinsed in PBS solution and developed by using Alex Fluor 594-conjugated secondary antibodies. For double labeling with Aβ antibodies, brain tissue sections were incubated overnight with anti-CD163 (1:100) or anti-α-smooth muscle actin (1:250), developed with the appropriate fluorescently conjugated secondary antibody (1:200), treated for 2 min with 70% formic acid, and washed in PBS solution before being incubated overnight with anti-$A\beta_{8-17}$ (1:100) or anti-$A\beta_{42}$ (1:100). Sections were rinsed in PBS solution, developed with the corresponding fluorescently conjugated anti-rabbit/mouse secondary antibody (1:200), and cover-slipped by using DakoShield Mounting Media (Dako). For anti-CD206 immunostaining, frozen brain sections were treated for 10 min in absolute EtOH, incubated overnight with anti-CD206 (1:100), and treated for 2 h with Alexa Fluor 594-conjugated anti-rat (1:200). The same sections were incubated overnight with either anti-Iba1 (1:200) or anti-GFAP (1:250) and developed with Alexa Fluor 488-conjugated secondary antibodies. Fluorescent photomicrographs were captured using a confocal microscope (Zeiss) and exported to Adobe Photshop CS.

Statistical Analysis. Images from singly labeled thioS, Ab8-17, and dextran-positive perivascular macrophages were serially quantified (3 sections per mouse) by using Openlab 4.0.2. imaging software (Improvision) by an examiner blinded to experimental treatment. ELISA measurements were done in triplicate for plasma, isolated blood vessels, and vessel-depleted cortical samples. For all treatment groups, mean values +/− SEM were used to construct histograms and analyzed by a one-way ot two-way Student's *t* test with significance set at $P < 0.05$.

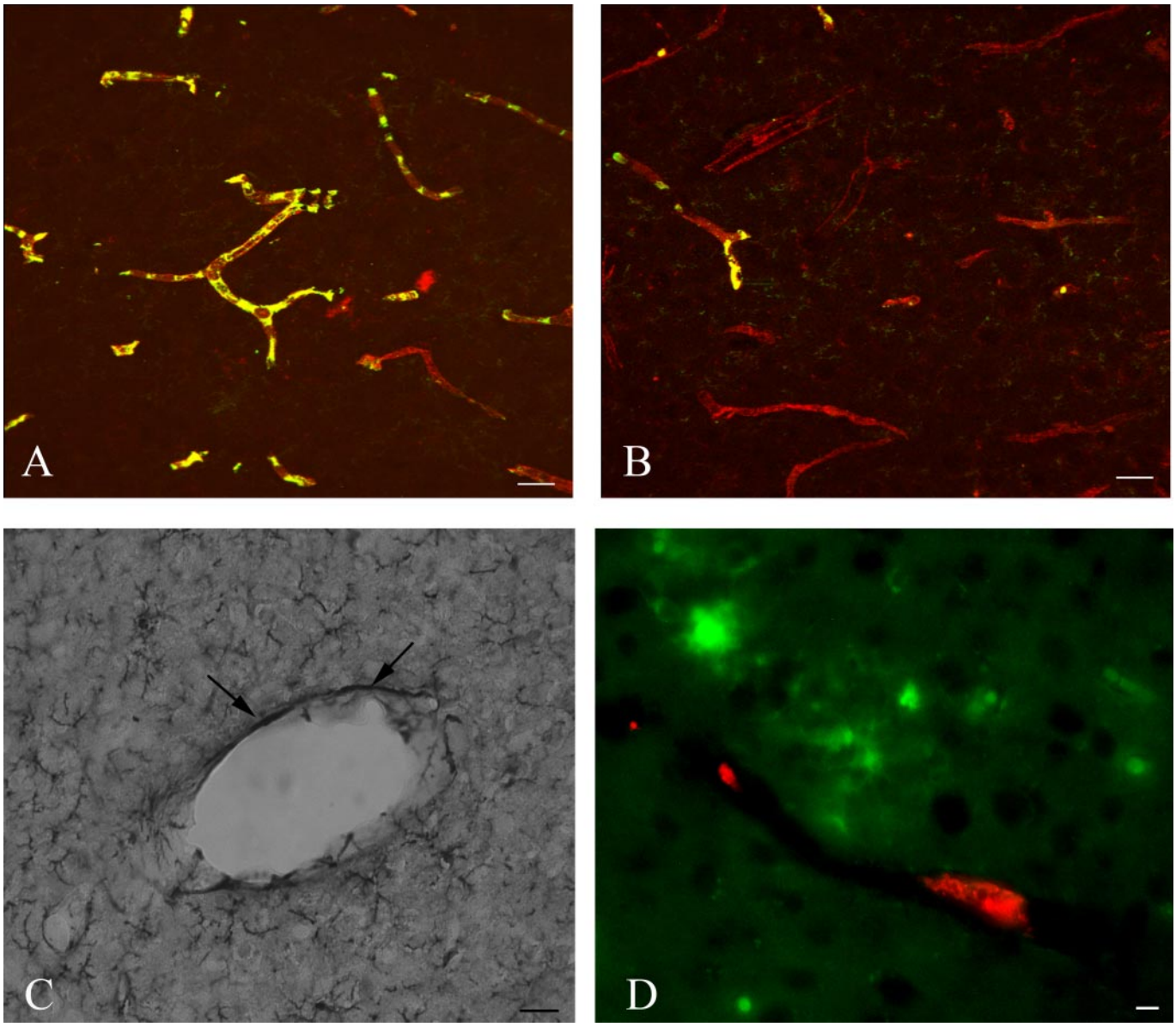

Fig. S1. Perivascular macrophages and juxtavascular microglia localize to cortical blood vessels. (*A* and *B*) Immunofluorescent photomicrographs depicting the specific localization of CD163-positive perivascular macrophages (green) to CD31-immunoreactive (red) cerebral blood vessels. Fewer perivascular macrophages were localized to perivascular spaces in the cortex of clodronate-treated (*B*) animals compared with vehicle-injected (*A*) TgCRND8 mice. (*C*) Iba-1-positive staining of juxta-vascular microglial processes abutting a cortical blood vessel (arrows). (*D*) Photomicrographs showing that CD206-immunoreactivity is expressed by perivascular macrophages (red), but not by Iba1-positive microglial cell bodies (green). (Scale bars: *A* and *B*, 35 μm; *C* and *D*, 20 μm.)

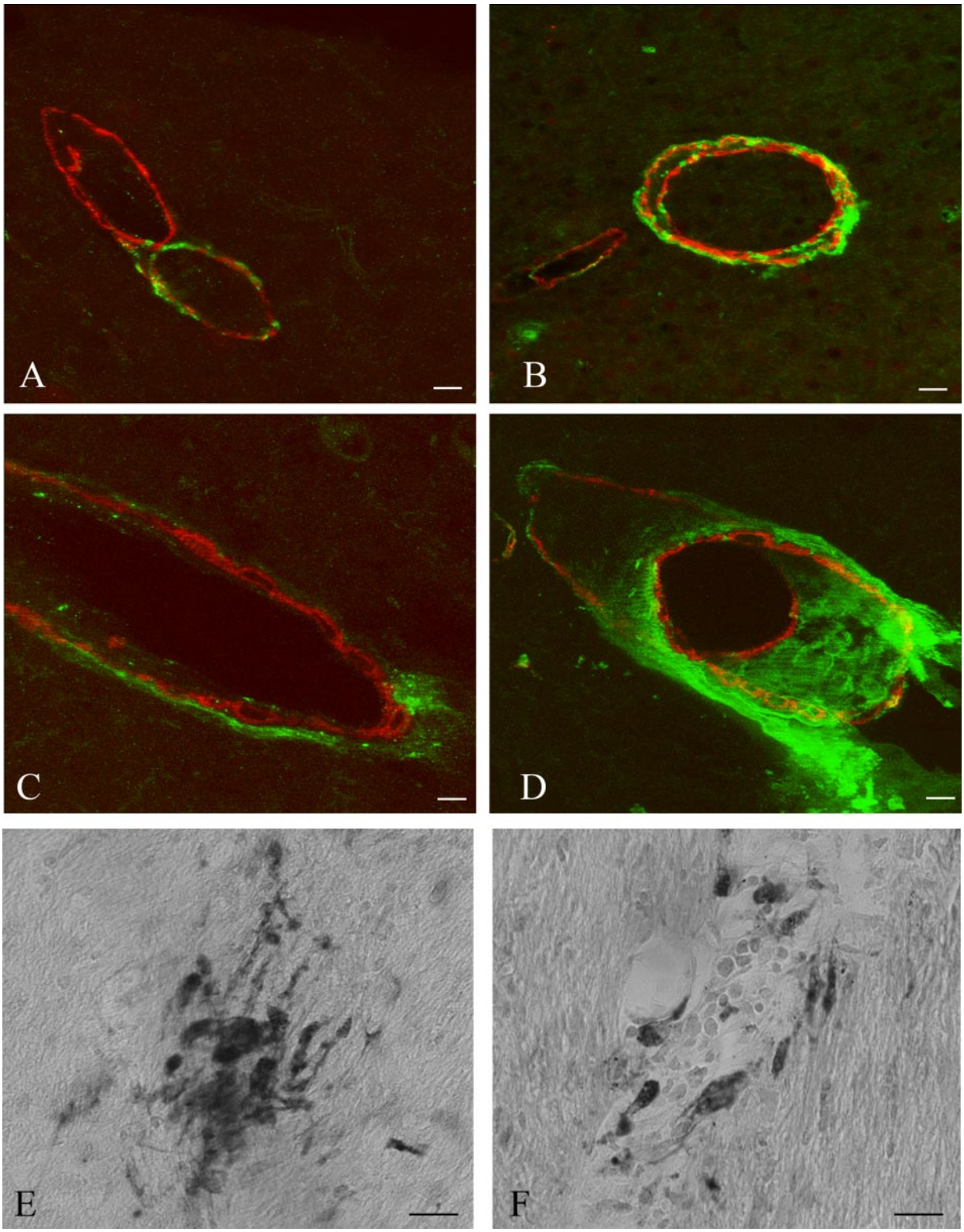

Fig. S2. Depletion of perivascular macrophages increases CAA load, but does not induce cerebral micro-hemorrhages. (*A–D*) Immunofluorescent photomicrographs showing double labeling of brain tissue sections from vehicle- (*A* and *C*) and clodronate-injected (*B* and *D*) animals stained for $A\beta_{8-17}$ (green) and α-smooth muscle actin (red). Note that the number and intensity of Aβ-positive cortical blood vessels was increased in mice treated with clodronate. (*E* and *F*) Tissue sections from the brains of mice treated with PBS- (*E*) or clodronate-containing liposomes (*F*) stained with Prussian blue to detect the presence of hemosiderin did not exhibit CAA-related micro-hemorrhages. (Scale bars: *A* and *B*, 5 μm; *C–F*, 20 μm.)

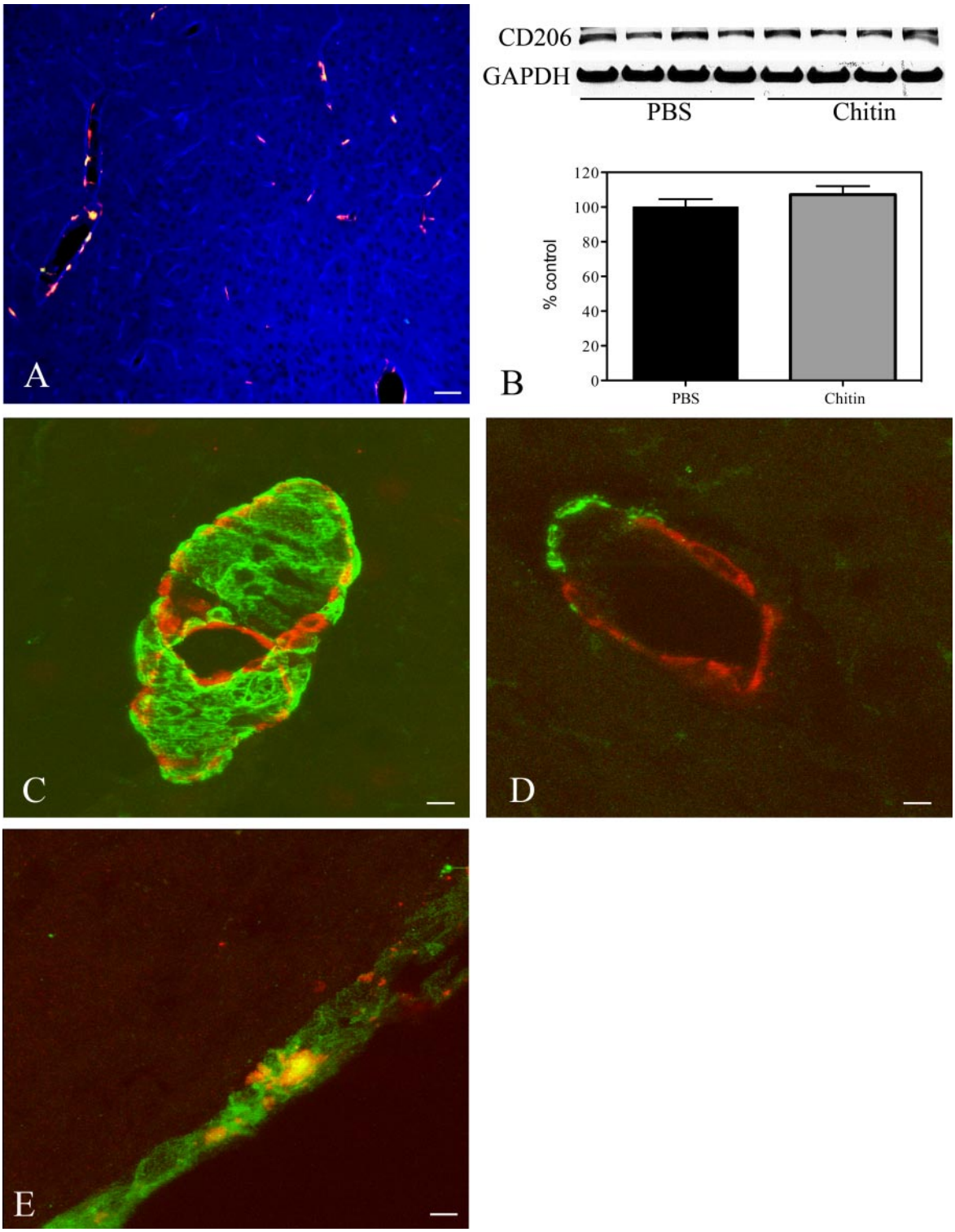

Fig. S3. Stimulation of perivascular macrophage turnover reduces Ab42 deposition in cortical blood vessels and leptomeninges. (*A*) Photomicrograph depicting the localization of dextran-positive perivascular macrophages (red and yellow) to the perivascular spaces of GLUT-1-immunoreactive cortical blood vessels (blue) in tissues sections from chitin-treated animals. (*B*) Quantification of CD206 levels in the right hemisphere of chitin-treated TgCRND8 mice did not differ significantly from vehicle-injected controls. (*C* and *D*) Immunofluorescent photomicrographs showing double labeling of brain tissue sections from vehicle- (*C*) and chitin-injected (*D*) animals stained for $A\beta_{8-17}$ (green) and α-smooth muscle actin (red). Note that the intensity of $A\beta$-labeling positive cortical blood vessels was decreased in mice treated with chitin. (*E*) CD163-immunoreactive macrophages (red) colocalized with $A\beta_{42}$-specific vascular amyloid (green) in chitin-injected mice.

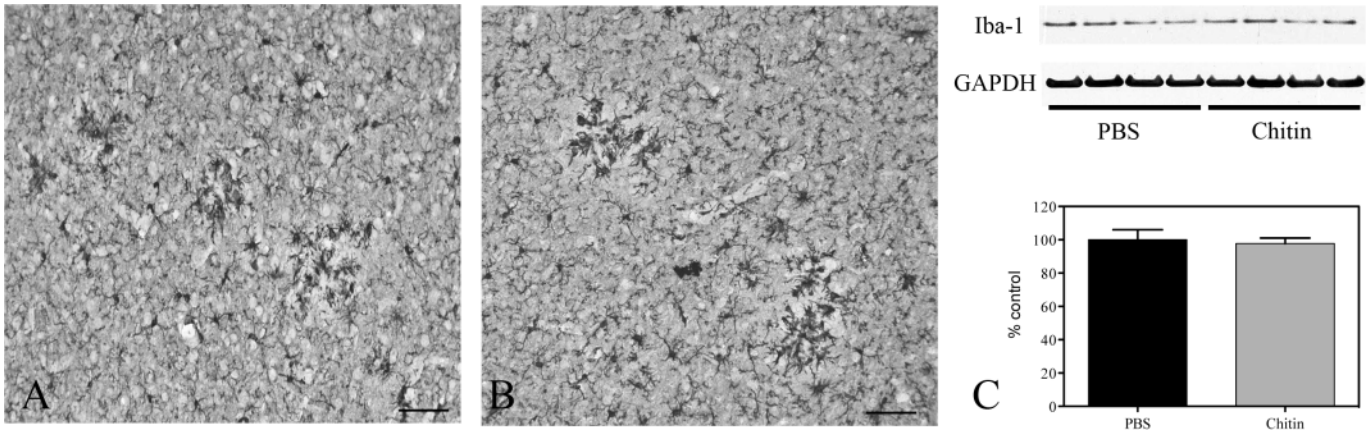

Fig. S4. Chitin treatment does not affect microglial activation. (*A*–*C*) Iba-1-positive microglia were noted throughout the cortex of vehicle- (*A*) and chitin-treated (*B*) mice. No differences were noted in Iba-1 levels (*C*) between treatment groups ($p = 0.91$, $n = 4$). Values represent mean ± SEM of samples analyzed in triplicte. (Scale bar, 70 μm.)