Supporting Information

Michaud et al. 10.1073/pnas.1215165110

SI Materials and Methods

Ligand Preparation. The monophosphoryl lipid A (MPL) immunostimulant was produced at GlaxoSmithKline from the LPS of *Salmonella minnesota* R595 following alkaline procedures, which have been described previously (1, 2). The MPL was dissolved in 2% glycerol (vol/vol) in PBS solution. LPS from *Escherichia coli* 055:B5 (Sigma) was dissolved in PBS.

NF-κB/AP-1 Activation Assay. Transfected HEK 293 cells (InvivoGen) with the expression vectors encoding human TLR4, MD-2, and CD14 were further stably transfected with the NF-κB reporter vector pNifty-2 secreted alkaline phosphatase (Invivogen). Cells were cloned by zeomycin (50 μg/mL) with further selection on the basis of appropriate TLR-stimulated expression of secreted alkaline phosphatase. Transfected cell lines were plated at 2×10^5 cells per well in a 24-well plate cultured in medium containing DMEM, 4.5 g/L glucose and L-glutamine, 10% FCS, and 0.5% penicillin/streptomycin. Cells were stimulated 24 h later for 5 h in FCS-free medium containing different concentrations of MPL and or PBS as negative control. To perform the TLR4 neutralization experiment, the functional anti-human TLR4 antibody (InvivoGen) was preincubated 1 h before the addition of the TLR ligands. Secreted alkaline phosphatase activity was measured using QUANTI Blue assay (InvivoGen).

Protein Extraction and Western Blot Analysis. The murine microglial cell line BV-2 was cultured at 37 °C in an H_2O-saturated and 5% CO_2 atmosphere in DMEM (Sigma) supplemented with 10% heat-inactivated FBS (Sigma), 100 U/mL penicillin (Sigma), and 100 μg/mL streptomycin (Sigma). All experiments were performed within less than 20 passages. BV-2 cells were plated in six-well plates in 10% FBS culture medium and allowed to reach 80% confluence. The medium was removed and replaced with serum-deprived DMEM. Afterward, cells were stimulated with MPL (1 μg/mL) or LPS (1 μg/mL). At the indicated time points, cells were put on ice, the medium was removed, and the cells were washed three times with ice-cold PBS with protease (Sigma) and phosphatase inhibitor mixtures (Sigma). Cells were then detached with a cell scraper in ice-cold ELISA lysis buffer [20 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1 mM disodium EDTA, 1 mM EGTA, 1% Triton X-100, 20 mM sodium pyrophosphate, 25 mM sodium fluoride, 1 mM β-glycerophosphate, 1 mM Na3VO4, and 1 μg/mL leupeptin; Cell Signaling Technology] supplemented with protease inhibitor mixture (Sigma), 1 mM PMSF (Sigma), and 1% phosphatase inhibitor mixture 3 (Sigma). The cell suspension was vortexed for 10 s at maximum speed, agitated for 10 min on ice, vortexed again for 30 s, and centrifuged at 13,000 × *g* for 20 min at 4 °C. Protein concentration was determined using the Quantipro BCA assay kit (Sigma) according to the manufacturer's protocol. Proteins were separated on a precast 10–20% SDS polyacrylamide Tris-Tricine gel (Bio-Rad). Separated proteins were then transferred onto PVDF membranes (PerkinElmer Life and Analytical Sciences) and detected by Western blotting. Blots were probed in 1 M Tris·HCl (pH 8.0), 5 M NaCl, 5% skim milk, and 0.05% Tween 20 with the following antibodies: IκBα (L35A5), β-actin antibodies (13E5), p-ERK1/2, ERK1/2, p-JNK1/2, JNK1/2, p-p38, p38, COX-2 (Santa Cruz), iNOS, α-Tubulin (BioLegend), and Arg-1 (all from Cell Signaling Technology if not otherwise stated). Blots were visualized using anti-mouse, anti-rabbit, or anti-goat secondary antibodies tagged with horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc.) and enhanced chemiluminescence detection system (GE Healthcare). Membranes were stripped in 25 mM glycine·HCl (pH 2.0) containing 1% SDS and probed as described above to allow the revelation of loading controls (nonphosphorylated kinases, β-actin, or α-Tubulin). Quantification was done by determining integrative density of the bands using a gel imaging system (Agfa Arcus II scanner and ImageJ software, version 1.32j) and background values were removed. Optical values were normalized according to the respective loading controls.

Cell Migration Assay. BV-2 cells were plated for 48 h in eight-well slide chambers (Falcon) in 10% FBS culture DMEM (Invitrogen) and allowed to reach 80% confluence. The medium was removed and replaced with low-serum-containing DMEM medium (1% FBS). Afterward, cells were stimulated with PBS or MPL (1 μg/mL) or LPS (1 μg/mL) and at the same time a scratch of a straight line was created at the middle of each well as previously described (3). Eighteen hours later, each well was stained for actin (phalloidin) and DNA (DAPI). The infiltrating cells in the 5-mm-long scratch were counted using a fluorescence microscope and Metamorph software (Leica).

Griess Assay. Nitrites were measured using the Griess Reagent Kit following the manufacturer's protocol (Invitrogen). BV-2 cells were incubated 24 h with MPL (1 μg/mL), LPS (1 μg/mL), or PBS. The medium (150 μL) was then transferred into a 96-well plate and mixed with 20 μL of Griess reagent and 130 μL of deionized water. The mixture was incubated 30 min at room temperature and the nitrite concentrations was determined by measuring the absorbance at 548 nm.

RNA Extraction and Real-Time PCR Analysis. BV-2 cells were incubated with control medium [DMEM supplemented with 5% heat-inactivated FBS (Gibco), 1 mM sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco)] or medium supplemented with 1 μg/mL MPL or 1 μg/mL LPS for the indicated time. Following incubation, cells were washed with ice-cold PBS and processed for RNA extraction using the RNeasy Mini kit, QIA shredder, and RNase-free DNase set (QIAGEN) accordingly to the manufacturer's instructions. RNA (450 ng) was then reverse-transcribed into cDNA using the RT First Strand Kit (SABiosciences and QIAGEN) and an equivalent amount of cDNA was amplified using iTaq SYBR Green Super Mix with ROX (BioRad) and specific primers for each gene analyzed. Each gene was amplified using the following pair of primers: β-actin (CACAGCTTCTTTGCAGCTCCTT; TGCCGGAGCCGTTGTC), SRA (TTGCTCTCTACCTCCTTGTGTTTG; CCATAGGACCTTGAGATGTGTCACT), TNFα (GAAACACAAGATGCTGGGACAGT; CATTCGAGGCTCCAGTGAATTC), CCL2 (CTGAAGCCAGCTCTCTCTTCCT; GAGCCAACACGTGGATGCT), TLR2 (GTACAGGGATCCGGGTGGTA; CGAGGCAAGAACAAAGAAAATGA), and 36B4 (ATAACCCTGAAGTGCTCGACATC; GGGTACCCGATCTGCAGACA). PCR reactions were performed on a ABI Prism 7900HT Sequence Detection System (Applied Biosystems) with initial cycles of 50 °C for 2 min and 95 °C for 3 min, followed by 45 cycles involving a denaturing step (95 °C, 15 s) and an annealing and extension step (58 °C, 45 s). Analysis was performed from data produced by the RQ Manager software (Applied Biosystems) using Microsoft Excel 2007 and GraphPad Prism 5.

***E. coli* Beads Phagocytosis Assay.** The phagocytic potential of BV-2 cells was assessed using the Vybrant Phagocytosis Assay following the manufacturer's protocol (Invitrogen). Briefly, BV-2

cells were incubated 18 h in six-well plates with MPL (1 μg/mL), LPS (1 μg/mL), or PBS. The medium was then replaced with fluorescein-coated *E. coli* K-12 BioParticles and incubated at 37 °C for 2 h. The cells were then suspended in 3.7% paraformaldehyde (PFA, pH = 7.4) with a cell scraper. The fluorescence intensity was measured for each condition on a FACS Aria II (BD Biosciences). The intensity of cells that were not incubated with BioParticles was used as a blank control and was subtracted to all values measured.

$A\beta_{1–42}$ Oligomer Preparation and Phagocytosis Assay. Oligomeric $A\beta_{1–42}$ was prepared as previously described (4). Briefly, lyophilized HiLyte Fluor 555-labeled $A\beta_{1–42}$ (Anaspec) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma) to 1mM, dried under vacuum, and stored at −80 °C. Immediately before use, $A\beta_{1–42}$ was dissolved in DMSO (Sigma) to 5 mM. Oligomeric $A\beta_{1–42}$ was prepared by adding ice-cold phenol red-free Ham's F-12 medium (Sigma) to obtain a final concentration of 100 μM. The solution was then incubated at 4 °C for 48 h. BV-2 microglia were plated at a density of 1×10^6 cells/mL. Before the addition of $A\beta_{1–42}$, cells were stimulated 18 h with 1 μg/mL of LPS or MPL. The cells were incubated with 1 μg/mL of fluorescent $A\beta_{1–42}$ oligomers in serum-deprived culture medium for 3 h at 37 °C. Cells were then washed three times with cold Dulbecco's PBS (DPBS) and collected for analysis by flow cytometry (FACS LSR II flow cytometer; BD Biosciences).

Immunofluorescence on Microglial Cells. BV-2 cells were plated on coverslips in six-well plates. Afterward, they were treated as described in the Aβ phagocytosis assays, but instead of being subjected to flow cytometry analysis they were fixed with 4% PFA and permeabilized in 0.2% Triton X-100 for phalloidin staining or with 0.1% saponin for Lamp-2. Cells were then blocked in 5% BSA and stained with 10 μg/mL phalloidin (Sigma), Lamp-2 (Stressgen), and DAPI (Invitrogen). Total BV-2 protrusion length was measured with the software ImageJ, version 1.47d. CHME cells were stained with Lysotracker Red (Invitrogen) following the manufacturer's instructions.

Quantification of Cytokine and Chemokine Levels in Mouse Sera. Blood cytokine and chemokine levels were measured in C57BL/6 mice 30–60 d old (Charles River) using ELISA (BD Bioscience) or a Luminex mouse cytokine-chemokine kit (MILLIPLEX; Millipore Inc.) following the manufacturer's protocols after the i.p. injection of either LPS (20 μg), MPL (50 μg), or PBS at 2 or 6 h postinjection. Blood cytokine and chemokine levels were also measured in the sera of APP_{swe}/PS1 mice that received 12 weekly i.p. injections of either LPS (3 μg), MPL (50 μg), or PBS. Three weeks after the last injection, the sera were collected and analyzed using the Cytometric Bead Array (BD Biosciences) according to the manufacturer's protocol.

FACS Analysis on Mouse Whole Blood and $A\beta_{1–42}$ Uptake by Monocytes. C57BL/6 female mice 6–8 wk old received an i.p. injection of a 200-μL volume of PBS, MPL (50 μg), or LPS (20 μg). Twenty-four hours following treatment, mice received 5 μg of fluorescent HiLyte Fluor 488-labeled $A\beta_{1–42}$ (Anaspec) via i.v. tail vein injection. Peripheral blood was harvested via cardiac puncture in the presence of lithium–heparin 2 h after i.v. injection of the Aβ HiLyte Fluor 488. Blood (250 μL) was aliquoted in a 96-well plate, which was then centrifuged to allow the separation and removal of the plasma. Cell pellets were resuspended twice in ammonium chloride-based buffer (Sigma) to eliminate red blood cells. Remaining cells were washed once with DPBS (Gibco) supplemented with 0.5% BSA, 5 mM EDTA, and 0.09% sodium azide, incubated for 10 min on ice in the presence of rat anti-mouse CD16/CD32 (clone 2.4G2, BD Fc Block; BD Biosciences), and further stained for 30 min with the following antibodies at their predetermined optimal concentration: a PE-conjugated antibody mixture containing hamster anti-mouse CD3 (clone 145-2C11), rat anti-mouse CD45R/B220 (clone RA3-6B2), rat anti-mouse Ly-6G (clone 1A8), and mouse anti-mouse NK1.1 (clone PK136), as well as APC-conjugated rat anti-mouse CD11b (clone M1/70) (BD Pharmingen), as previously described (5). Cells were fixed for 15 min with a 2% PFA solution in PBS. Samples were acquired on a flow cytometer (BD FACSCanto II) and data analyzed with the FACSDiva software (BD Biosciences). Monocytes were identified by their side/forward scatter properties, excluding debris and doublets, and were gated as CD3-/CD45R/B220-/Ly-6G-/NK1.1-(Lineage-)/CD11b+ cells (5). Aβ uptake was assessed by reporting the percentage and mean fluorescence intensity (geometric mean) of positive HiLyte fluor 488 Aβ1–42 cells among gated monocytes. Flow cytometry analysis of whole blood was also performed in APP_{swe}/PS1 mice that received 12 weekly i.p. injections of either MPL (50 μg), LPS (3 μg), or PBS. The procedure was similar to that described previously except that red blood cells were lysed with BD Pharm Lyse (BD Biosciences); the antibodies used were anti-mouse CD45-V500 (BD Biosciences), CD11b-A700 (eBioscience), CD115-APC (eBioscience), and Ly6G-PE (BD Biosciences); and cells were not fixed. Samples were acquired on a flow cytometer (BD LSR II) and data analyzed with FlowJo software, version 10.

Transgenic Mouse Lines and Treatment. APP_{swe}/PS1 transgenic mice harboring the human presenilin I (A246E variant) and the chimeric mouse/human Aβ precursor protein ($APP695_{swe}$) under the control of independent mouse prion protein (PrP) promoter elements [B6C3-Tg(APP695)3Dbo Tg(PSEN1)5Dbo/J] (Jackson ImmunoResearch Laboratories, Inc.) were maintained in a C57BL/6J background and acclimated to standard laboratory conditions. Only males were used in the experiments. All protocols were conducted according to the Canadian Council on Animal Care guidelines, as administered by the Laval University Animal Welfare Committee. MPL (50 μg/mouse, 130 μL), LPS (3 μg/mouse, 130 μL), or PBS (130 μL) was administered once a week by i.p. injection in 3-mo-old APP_{swe}/PS1 mice for 12 consecutive weeks.

Tissue Collection. To collect brain tissues, mice were deeply anesthetized via an i.p. injection of a mixture of ketamine hydrochloride and xylazine and then perfused intracardially with ice-cold 0.9% saline. Brains were rapidly removed from the skulls and placed in cold PBS solution and hemibrains were then separated. One hemibrain was rapidly frozen in liquid nitrogen and stored at −80 °C for protein analysis. The other one was postfixed for 2–4 d in 4% PFA (pH 7.4) at 4 °C and then placed in a PFA solution containing 10% sucrose overnight at 4 °C. The frozen brains were mounted on a microtome (Leica) and cut into 25-μm coronal sections. The slices were collected in cold cryoprotectant solution [0.05 M sodium phosphate buffer (pH 7.3), 30% ethylene glycol, and 20% glycerol] and stored at −20 °C until immunocytochemistry or in situ hybridization histochemistry.

TLR2 and TNF-α in Situ Hybridization. Every 12th section of brain slices was mounted on Colorfrost/Plus microscope slides (Fisher Scientific), starting from the end of the olfactory bulb to the end of the cortex. In situ hybridization histochemical localization of TLR2 and TNF-α was performed using ^{35}S-labeled cRNA probes. Plasmids were linearized and sense and antisense cRNA probes were synthesized with appropriate RNA polymerase. All plasmids were analyzed for sequence confirmation and orientation. Riboprobe synthesis and preparation as well as in situ hybridization were performed according to a protocol described previously (6). All images were captured using a Nikon Eclipse 80i microscope equipped with a digital camera (QImaging).

Aβ Plaques Immunofluorescence and Stereological Analysis. To stain Aβ plaques, free-floating sections were treated 30 min with a permeabilization/blocking solution containing 0.4% Triton X-100, 4%

goat serum, and 1% BSA (Sigma-Aldrich) in potassium PBS (KPBS). Using the same solution, sections were immunostained 60 min with monoclonal anti-Aβ (6E10, 1:3,000; Covance), washed three times for 5 min in KPBS, and then incubated with secondary antibody goat anti-mouse Cy3 conjugated (1:1,000; Jackson ImmunoResearch Laboratories, Inc.) for 60 min. Stereological analysis was performed as described previously (7). The contours of the cortex areas were traced as virtual overlay on the steamed images. The number of plaques in these regions and the area occupied by all Aβ-labeled plaques were determined.

Generation of Chimeric Mice. Bone-marrow cells were obtained by flushing femur from $GFP^{+/-}$ donor mice (6 wk of age) in a sterile environment. Cells were harvested using DPBS (Wisent) complemented with 2% FBS. To remove clumps from the extract, the preparation was filtered using a 40-μm nylon filter and pelleted. The supernatant was removed and cells resuspended in DPBS before being chased through a 25-g syringe needle. Cells were centrifuged and resuspended in fresh DPBS. Two-month-old receiving APP_{swe}/PS1 mice were exposed to 5-Gy total-body irradiation using a cobalt-60 source (Theratron-780; MDS Analytical Technologies). Twenty-four hours later, the animals were injected via tail vein (200 μL) with 1×10^7 bone marrow cells freshly collected and purified from $GFP^{+/-}$ donor mice. Irradiated mice transplanted with bone marrow cells were housed in sterile cages and treated with antibiotics (0.2 mg/mL trimethorpine and 1 mg/mL sulfamethoxazole in drinking water was given 7 d before and up to 2 wk after irradiation). The bone marrow reconstitution procedure was confirmed 6 wk later by flow cytometry analysis of GFP-positive blood cells. GFP-positive cells in the brains of these mice were quantified by stereology as described previously (7).

Protein Extraction from Brain Samples and Western Blot Analysis. The extraction of protein from brain hemispheres was performed on ice to minimize protein degradation. One hemisphere was placed in a 1-mL syringe connected with a 20-gauge needle. Five hundred microliters of buffer [50 mM Tris·HCl (pH 7.6), 0.01% Nonidet P-40, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1 mM PMSF, and protease inhibitor mixture (Roche)] were added and the brain sample was passed 10 times through the needle, which homogenizes the tissue in the buffer. Samples were then centrifuged for 10 min at 855 × g at 4 °C. The supernatant was then collected and protein concentration was determined using the BCA protein assay (Pierce) accordingly to the manufacturer's protocol. Monomeric Aβ was detected by immunoblotting as follows. Forty micrograms of brain protein extract were separated on a precast 10–20% polyacrylamide Criterion Tris-Tricine gel (Bio-Rad). Separated proteins were then transferred onto PVDF membranes (PerkinElmer). Membranes were incubated in DPBS (Gibco) at 90 °C for 10 min. Membranes were then incubated for 1 h in blocking solution, which consisted of TBS-T [50 mM Tris·HCl (pH 8.0), 150 mM NaCl, and 0.05% Tween 20] supplemented with 1% BSA (Sigma) and 5% nonfat dry milk and probed for 16 h at 4 °C with a mouse anti-amyloid β protein monoclonal antibody (clone 6E10; Covance) diluted to 1:1,000 in TBS-T supplemented with 5% BSA and 0.02% sodium azide. Membranes were washed once for 15 min and three times for 5 min in TBS-T at room temperature. Between each washing step, membranes were rinsed three times with TBS-T. Membranes were then incubated for 1 h at room temperature in blocking solution supplemented with goat anti-mouse IgG HRP (Jackson ImmunoResearch Laboratories, Inc.) diluted at 1:10,000. Membranes were washed and rinsed as described above before being washed for 5 min in TBS [50 mM Tris·HCl (pH 8.0) and 150 mM NaCl] and rinsed four times with Milli-Q water. Localization of the HRP signal on the membrane was revealed using Super Signal West Dura Extended Duration Substrate (Thermo Scientific) according to the manufacturer's instructions and revealed on Amersham Hyperfilm (GE Healthcare). To detect β-actin, membranes were stripped in Reblot Plus Strong solution (Millipore) accordingly to the manufacturer's recommendation and incubated in blocking solution for 1 h. Membranes were then incubated for 16 h at 4 °C in anti–β-actin rabbit monoclonal antibody (13E5; Cell Signaling Technology) diluted at 1:2,000 in TBS-T supplemented with 5% BSA and 0.02% sodium azide. Membranes were washed, incubated with goat anti-rabbit IgG HRP (H+L) (Jackson ImmunoResearch Laboratories, Inc.) diluted at 1:5,000 in blocking buffer, washed, and revealed as described above. Films were digitalized using a Scanjet 4370 (Hewlett-Packard) and signal intensity was measured using ImageJ (version 1.44p).

Isolation of Brain Leukocytes and FACS Analysis. Ten-month-old APP_{swe}/PS1 mice received five consecutive daily i.p. injections of MPL (25 μg) or PBS. Mice were deeply anesthetized via an i.p. injection of a mixture of ketamine hydrochloride and xylazine and then perfused intracardially with ice-cold DPBS. Brains were homogenized in DMEM supplemented with 10 mM Hepes and 2% FBS and filtered through a 70-μm filter. The cell suspension was then centrifugated at 300 × *g* for 10 min at room temperature. After centrifugation, the supernatant was aspirated and cells were gently resuspended in 1 mL of 37% Percoll. The cell suspension was then underlaid with 70% Percoll and centrifugated at 600 × *g* for 40 min with minimal acceleration and deceleration. The cell ring at the interphase was then collected and mixed thoroughly with DPBS and 2% FBS. The solution was then centrifuged at 300 × *g* for 10 min and washed with DPBS + 2% FBS twice. Cells were first incubated on ice for 15 min with purified rat anti-mouse CD16/CD32 (Mouse BD Fc Block; BD Biosciences). The mix was then incubated on ice with CD45-PE (BD Biosciences) for 35 min and then washed with DPBS + 2% FBS. For intracellular Aβ detection, cells were permeabilized and fixed with BD Cytofix/Cytoperm Plus following the manufacturer's protocol (BD Biosciences). Cells were then resuspended and incubated 60 min with 6E10 (1:100; Covance). The cells were then washed and incubated with IgG anti-mouse Alexa-Fluor 488 (Invitrogen). The cells were analyzed using a two-laser, six-color FACS Canto II flow cytometer and data acquisition was done with BD Facs Diva software (Version 6.1.2; BD Biosciences). Cells were then acquired according to the different fluorescent antibodies. Results were analyzed using FlowJo software (Tree Star).

Behavioral Analysis. Mice were housed one per cage and acclimated to standard laboratory conditions (12-h light, 12-h dark cycle; lights on at 0700 hours and off at 1900 hours) and tested during the "light on" phase of the day. Food and water were provided for ad libitum consumption. The behavioral experimenter was blinded to the treatment status of animals. The behavioral and general health of mice was monitored using a modified version of the primary observation screen described by the SHIRPA protocol (8).

Left–Right Discrimination Learning. To assess hippocampal-dependent spatial learning and memory, mice were trained in the T water maze (TWM). The T-maze apparatus (length of stem 64 cm, length of arms 30 cm, width 12 cm, height of walls 16 cm) was made of clear fiberglass and filled with water (23 ± 1 °C) at a height of 12 cm. An escape platform (11 × 11 cm) was placed at the end of the target arm and was submerged 0.5 cm below the surface. The acquisition phase allows the assessment of APP_{swe}/PS1 mice for left–right spatial learning. During the first two trials, platforms were placed on each arm of the TWM. To assess arm preferences, the least-chosen arm was reinforced by water escape. The mice were placed in the stem of the TWM and chose to swim either left or right until they found the submerged platform and escaped to it, for a maximum of 60 s. After reaching the platform, the mice were allowed to stay on it for 20 s and then were immediately placed back into the maze. If

the animals did not find the platform within this time limit, they were gently guided onto it. Repeated trials were presented on the same day up to a maximum of 48 trials. A rest period of at least 10–15 min intervened between each block of 10 trials. A mouse was considered to have learned the task when it made no errors in a block of five consecutive trials. The reversal learning phase was then conducted 48 h later. During this phase, the same protocol was repeated except that the mice were trained to find the escape platform on the side opposite that they had learned during the acquisition phase. Repeated trials were presented on the same day up to a maximum of 24 trials. Two measures were taken: number of trials to reach the criterion (five of five correct choices made on consecutive trials) and escape latency. The experimenter was blind to the treatments, and all mice were distributed randomly. After 10 trials in the TWM, mice were dried and given a rest period to prevent hypothermia and fatigue.

Statistical Analysis. For the chronic treatment experiments in APP_{swe}/PS1 mice, the Shapiro-Wilk test was used to confirm normality of the data. The ANOVA and Tukey's tests were applied to identify differences between treatment groups in the APP-PS1 model. Statistical significance was assigned at the value for $P \leq 0.05$. For the other experiments, statistical analysis was achieved using one-way ANOVA and Student t test, homogeneity of variance was performed with Levene's test, and $P < 0.05$ was set as the level of a significant difference. Statistical analysis was performed using the Unistat and SPSS softwares.

1. Baldridge JR, Crane RT (1999) Monophosphoryl lipid A (MPL) formulations for the next generation of vaccines. *Methods* 19(1):103–107.
2. Ulrich JT, Myers KR (1995) Monophosphoryl lipid A as an adjuvant: Past experiences and new directions. *Pharm Biotechnol* 6:495–524.
3. Liang C-C, Park AY, Guan J-L (2007) In vitro scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc* 2(2):329–333.
4. Stine WB, Jr., Dahlgren KN, Krafft GA, LaDu MJ (2003) In vitro characterization of conditions for amyloid-beta peptide oligomerization and fibrillogenesis. *J Biol Chem* 278(13):11612–11622.
5. Mildner A, et al. (2007) Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci* 10(12):1544–1553.
6. Laflamme N, Lacroix S, Rivest S (1999) An essential role of interleukin-1beta in mediating NF-kappaB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. *J Neurosci* 19(24):10923–10930.
7. Simard AR, Soulet D, Gowing G, Julien J-P, Rivest S (2006) Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* 49(4):489–502.
8. Rogers DC, et al. (1997) Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mamm Genome* 8(10):711–713.

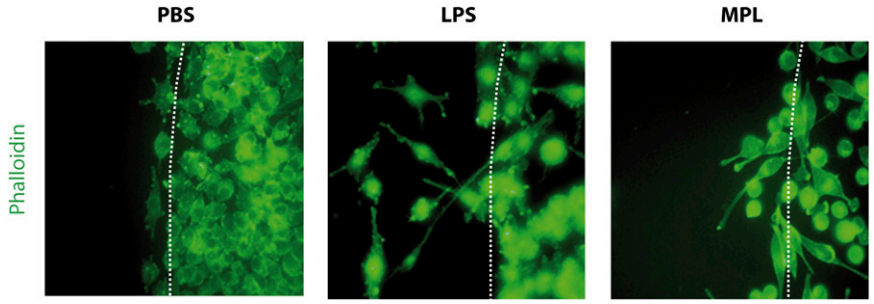

Fig. S1. Increased microglial cell motility following MPL treatment. Migration of BV-2 cells was assessed using the scratch assay test. Cells were incubated with MPL (1 μg/mL), LPS (1 μg/mL), or PBS and at the same time a straight line was scratched in the middle of each well. Eighteen hours later, each well was stained for actin (phalloidin), as illustrated by these representative examples. We observed a higher number of cells migrating within the scratched area after stimulation with LPS or MPL. Moreover, longer filopodia spreading into the clear area were observed for the LPS- or MPL-treated cells.

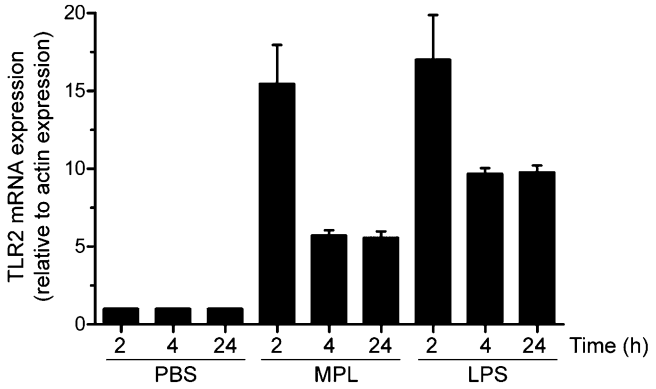

Fig. S2. TLR2 mRNA expression in microglia following MPL or LPS stimulation. BV-2 microglia were incubated with MPL (1 μg/mL), LPS (1 μg/mL), or PBS for 2, 4, or 24 h ($n = 3$). TLR2 mRNA was quantified by real-time PCR analysis. Data are expressed as the means ± SEM.

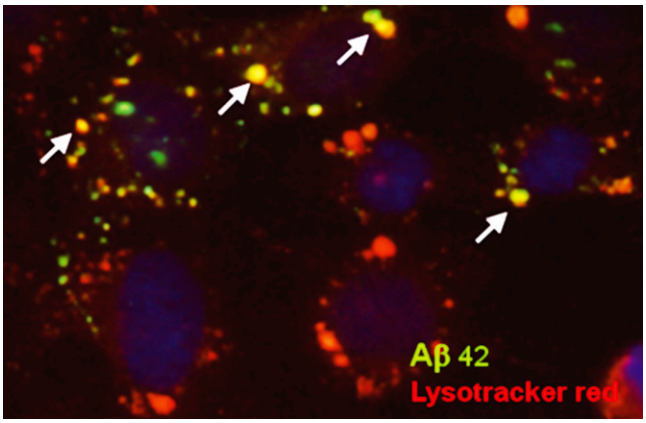

Fig. S3. Aβ internalization within lysosomes following MPL treatment in microglial cell line. CHME human microglia were stimulated with 1 μg/mL of MPL for 18 h and then incubated 3 h with fluorescent $A\beta_{1-42}$. Lysosomes were stained with LysoTracker Red and the nucleus with DAPI (blue). The white arrows on this representative picture of fluorescence microscopy show the localization of $A\beta_{1-42}$ (green) within microglia lysosomes (red) after MPL treatment.

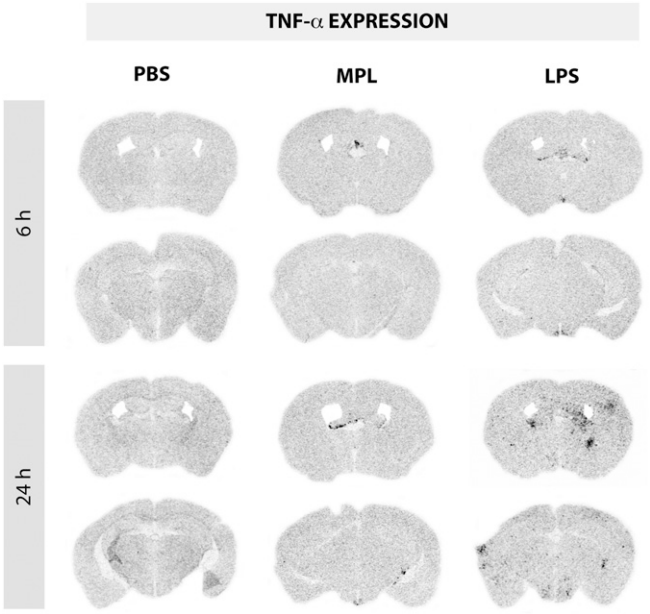

Fig. S4. Mild induction of TNF-α expression in the brains of MPL-injected mice. Coronal sections showing representative examples of TNF-α mRNA in situ hybridization in brains of WT mice following an acute i.p. injection of PBS, MPL (50 μg), or LPS (3 μg). Twenty-four hours after the injection, LPS provoked a strong TNF-α induction in the circumventricular organs, choroid plexus, and brain parenchyma, whereas MPL triggered only a weak TLR2 induction in the circumventricular organs but not in the brain parenchyma.

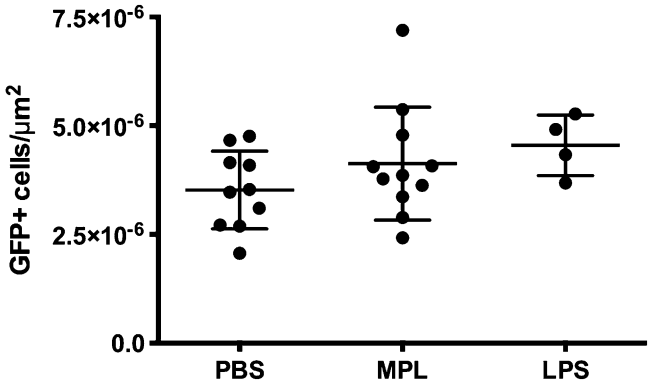

Fig. S5. Bone marrow-derived cell number is not increased in the brains of APP_{swe}/PS1 mice injected weekly with MPL. Six-week-old APP_{swe}/PS1 mice were irradiated with 5 Gy and transplanted 24 h later with 10 million $GFP^{+/-}$ bone marrow cells. At 3 mo of age, MPL (50 μg), LPS (3 μg), or PBS was administered once a week by i.p. injection for 12 consecutive weeks. Three weeks after the last injection, the number of GFP^{+} cells in the whole brain was quantified by stereology (n = 10 for PBS, n = 11 for MPL, n = 4 for LPS). Each point represents a single mouse and the horizontal bars are the mean for each group ± SD.

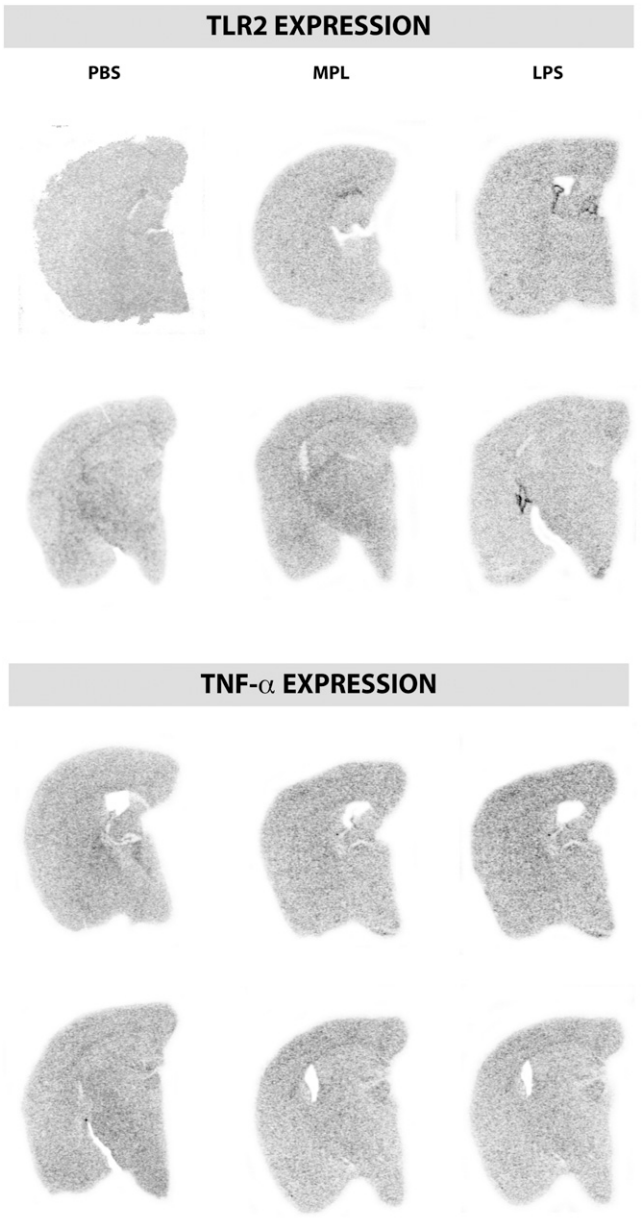

Fig. S6. TLR2 and TNF-α expression in APP_{swe}/PS1 mice is not sustained 3 wk after 12 weekly MPL injections. MPL (50 μg), LPS (3 μg), or PBS was administered once a week by i.p. injection in 3-mo-old APP_{swe}/PS1 mice for 12 consecutive weeks. Coronal sections showing representative examples of TLR2 and TNF-α mRNA in situ hybridization in the brain of these mice 3 wk after the last injection. No TLR2 or TNF-α induction remained in the MPL group. In the LPS group, TNF-α expression also vanished but a very weak TLR2 signal could be observed in periventricular regions.

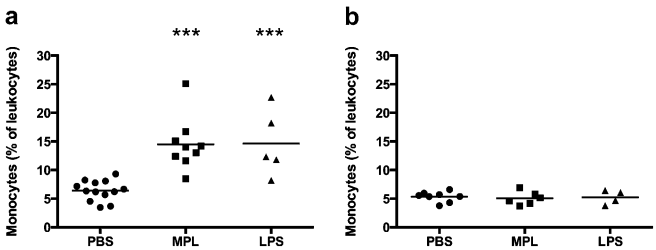

Fig. S7. MPL triggers the expansion of blood monocytes in APP_{swe}/PS1 mice injected weekly with MPL. MPL (50 μg), LPS (3 μg), or PBS was administered once a week by i.p. injection in 3-mo-old APP_{swe}/PS1 mice for 12 consecutive weeks. Their blood was analyzed (*A*) 24 h and (*B*) 6 d later by flow cytometry. Monocytes were considered as $CD45^+CD11b^+CD115^+Ly6G^-$. Data are expressed as the percentage of total blood leukocytes excluding debris. ***$P < 0.001$ (vs. PBS).

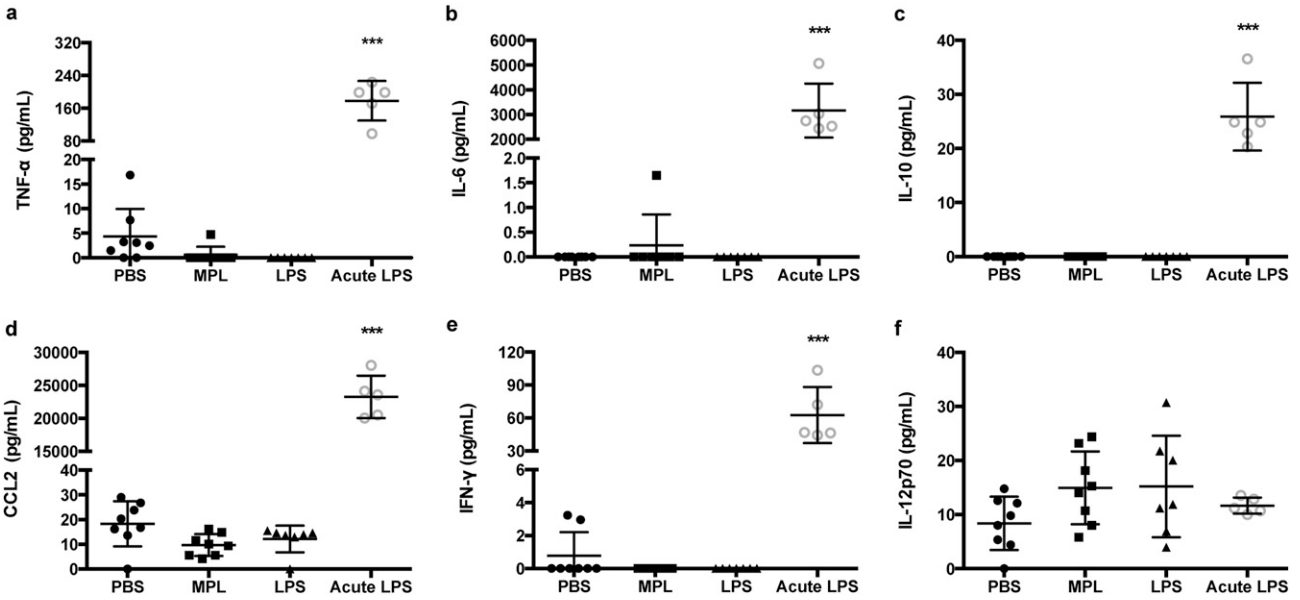

Fig. S8. MPL does not induce a sustained peripheral innate immune activation after repeated injections in APP_{swe}/PS1 mice. MPL (50 μg), LPS (3 μg), or PBS was administered once a week by i.p. injection in 3-mo-old APP_{swe}/PS1 mice for 12 consecutive weeks. Three weeks after the last injection, (*A*) TNF-α, (*B*) IL-6, (*C*) IL-10, (*D*) CCL2, (*E*) IFN-γ, and (*F*) IL-12p70 levels were quantified in their sera by Cytometric Bead Array ($n = 7–8$). As a positive control (Acute LPS), the sera of wild-type mice 6 h after a single acute LPS (3 μg) i.p. injection were quantified ($n = 5$). Each point represents a single mouse and the horizontal bars are the mean for each group ± SD ***$P < 0.001$ (vs. PBS, MPL, and LPS).

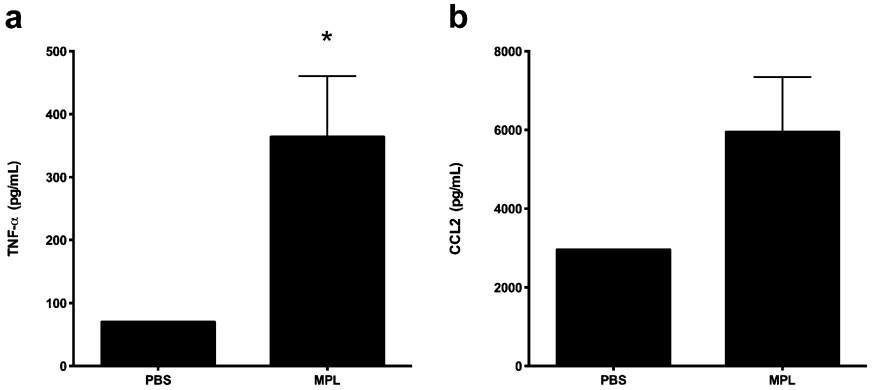

Fig. S9. MPL retains its ability to stimulate the peripheral innate immune system after repeated injections in APP_{swe}/PS1 mice. MPL (50 μg) or PBS was administered once a week by i.p. injection in 3-mo-old APP_{swe}/PS1 mice for 13 consecutive weeks. TNF-α (*A*) and CCL2 (*B*) in the sera of these mice (2 h post-injection) were quantified by ELISA ($n = 6–7$). Data are expressed as the means ± SEM. *$P < 0.05$ (vs. PBS).