***Supplementary Information***

***Materials and Methods***

**Animals**

APP mice have a genetic mutation in the amyloid precursor protein (APP) that results in significant increases in Aβ40 and Aβ42 in the plasma and the brain by three to five months (Lanz et al., 2004) and Aβ plaques in the brain by eight months (Kawarabayashi et al., 2001). These mice show deficits in learning and memory tasks by nine months of age as compared to age-matched controls (Hsiao et al., 1996; King et al., 1999). Male APP mice were back-crossed to the B6/129 strain. Genotyping was performed with DNA samples prepared from each individual mouse pup tail tip, and genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). PCR was used to identify APP mice using the methods described previously (Hsiao et al., 1995).

**Plasmid construction and AAV2/1 generation**

For pAAV2-TRE-CD200 construction, a PCR fragment containing murine full-length CD200 (CD200FL; referred to as CD200 in this article) coding sequence was amplified using the primers: CD200 Fw: 5'-GGCGGATCCGCCACCATGGGCAGTCTGGTATTC-3' and Rev: 5'-GGGCTCGAGTTATTTCATTCTTTGCATCCC-3' and murine cDNA (clone 6413363, Open Biosystems, GE Dharmacon, Lafayette, CO) as a template DNA, digested with *Bam* HI and *Xho* I and inserted into the MCS of pAAV2-TRE-MCS-WPRE.

**Stereotactic Injection**

At six months of age, mice were anesthetized with isoflurane and immobilized in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). An incision was made over bregma, and a 1-mm burr hole was drilled through the skull 2.1 mm posterior and 1.6 mm lateral to bregma on each side. 2 µl per brain of AAV2/1-TRE-GFP (2 x $10^9$ VP) or AAV2/1-TRE-CD200 (2 x $10^9$ VP) and AAV2/1-CMV-tTA (2 x $10^9$ VP) in saline was injected 1.8 mm below the skull surface into the CA1 region at a rate of 0.5 µl/min using a 10 µl Hamilton syringe and an automated microinjector (Stoelting, Wood Dale, IL). The needle was removed from the brain 5 minutes after each injection to allow for diffusion of the virus.

**Tissue Preparation**

After deep anesthetization with i.p. injection of a ketamine/xylazine cocktail (100 mg/kg / 20 mg/kg, respectively), mice were perfused transcardially with phosphate-buffered saline (PBS). For ELISA, hippocampi were rapidly removed and frozen on dry ice. For immunohistochemistry, PBS perfusion was followed by transcardial perfusion with 4% paraformaldehyde (PFA). The brain was then rapidly removed and immersed in 4% PFA followed by 24-hour serial dilutions in sucrose. Brains were mounted in cryoprotectant cutting medium and serially sectioned coronally at 30 µm using a Leica cryostat (Leica Biosystems, Bannockburn, IL).

**Immunohistochemistry**

Sections were placed in 80°C tri-sodium citrate for antigen retrieval followed by permeation with 0.2% Triton X-100. Blocking was performed for one hour with 2% donkey or normal goat serum. For BrdU staining, sections were first incubated for 1.5 hours in a 50% formamide/50% 2xSSC solution at 65°C followed by a 10 minute rinse in 2xSSC. Sections were then incubated for 30 minutes in 2M hydrochloric acid and rinsed for 10 minutes in 0.1M borate buffer before permeation and blocking steps. Primary antibody incubation was performed overnight at 4°C in blocking buffer using specific antibodies against CD200, BrdU, doublecortin (Dcx), nitrogen oxygen synthase-2 (NOS2), chitinase-3-like-3 (YM1), F4/80, P2ry12, CD169, Iba-1, and pan-Aβ. Antibody dilutions and sources are described in **Table S1**. For immunofluorescence, antigens were visualized using Alexa Fluor®488-, 594-, or 647-conjugated anti-rat, anti-goat, or anti-rabbit IgG (1:600 each, Invitrogen, Carlsbad, CA). This was followed by total nuclei staining using Hoechst 33342 (1:10,000, AnaSpec, Inc, San Jose, CA). For non-fluorescent immunohistochemistry, primary antibody incubation was followed by biotinylated anti-rat IgG or anti-rabbit IgG Envision Plus (DAKO, Carpinteria, CA) and streptavidin/biotin complex formation using a commercially available kit (Vector Labs, Burlingame, CA). Antibody binding was visualized with 3,3'-diaminobenzidine labeling (DAB kit, Vector Laboratories, Burlingame, CA). For Aβ staining, immunodetection was followed by counterstaining of compact plaques with 0.1% Thioflavin-S in 50% ethanol in PBS (Sigma-Aldrich, St. Louis, MO).

**Cell Culture**

For cytokine stimulation, primary neurons were plated in a 6-well plate at 1,600,000/well and treated with conditioning medium including mouse IL-10 or IL-4 for 24 hours then lysed with lysis buffer (10 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 2 mM $Na_3VO_4$, 1 % Triton X-100, 10 % glycerol, 0.1 % SDS, 0.5 % deoxycholate, and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO)). For Ab stimulation, neurons in a 24-well plate at 400,000/well were treated with oligomeric Ab42 (100 μM, prepared by resolving lypophilized Aβ peptides (Invitrogen, Carlsbad, CA) in hexafluoro-2-propanol; dried peptides were dissolved in dimethyl sulfoxide at 5mM and diluted in PBS before 24-hour incubation at 4°C) in the absence or presence of IL-4 (2 ng/ml) and harvested with lysis buffer.

AAV-293 cells were maintained in complete DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen, Carlsbad, CA) in the presence of 5% $CO_2$. For AAV2/1 transduction, cells were seeded at 100,000 (AAV-293) or 200,000 (primary neurons)/well in tissue culture media in a 48-well plate. AAVs were added directly into the media and incubated for 14 days before lysis, sonication, and immunoblotting.

For the co-culture neural differentiation study, neural stem cells were plated onto poly-D-lysine- and laminin-coated coverslips (Sigma-Aldrich, St. Louis, MO) in 24-well plates at 100,000/well. Microglia were subjected to one-hour pre-treatment with soluble CD200Fc ligand (100 ng/ml), and then plated at 10,000/well in transwell inserts (Corning, Inc, Corning, NY) for indirect co-culture with neural stem cells for seven days.

To determine the effects of CD200 stimulation on microglial phagocytosis of Aβ42, primary microglia were plated at 0.2 x $10^6$ per well in a 12-well plate for n= 3 per

group. After plating, microglia were pre-treated with 5 μM untagged Aβ42 (rPeptide, Bogart, GA) for 18 hours in fresh serum-free DMEM with 1% Penicillin/ Streptomycin (Invitrogen, Carlsbad, CA). Aβ42 was provided as ultra pure peptide pre-treated with hexafluoroisopropanol (HFIP) from the manufacturer and resuspended in 1% $NH_4OH$ at 1 mg/ml prior to resuspension in buffer for experimentation, as recommended by the manufacturer. Microglia were then treated with 100 ng/ml soluble CD200Fc ligand for one hour prior to stimulation with 5 μM fluorescein isothiocyanate (Fitc)-tagged Aβ42 ($A\beta42^{Fitc}$) for 30 minutes to allow for phagocytosis. $A\beta42^{Fitc}$ was fibrillized prior to stimulation by spinning overnight at room temperature on a magnetic stir plate. We previously showed that uptake of oligomeric Aβ42 by mononuclear phagocytes is neglible compared to uptake of fibrillar Aβ42 (Yamamoto et al., 2008). This ensures that any reductions in phagocytosis of $A\beta42^{Fitc}$ were not due to prior consumption of Aβ42 during pre-treatment. After 30 minutes, microglia were trypsinized and prepared for flow cytometric analysis.

**Immunocytochemistry**

Cells were permeabilized with 1% Triton X-100 in PBS followed by blocking in 5% normal goat serum and primary antibody incubation overnight. Primary antibodies include specific antibodies against mature neuronal marker, β-III tubulin (β3T), astrocyte marker, glial fibrillary acidic protein (GFAP), and anti-oligomeric Aβ (NU-1) (Lambert et al., 2007). Antibody dilutions and sources are outlined in **Table S1**. Immunofluorescence was visualized using Alexa Fluor®488- or 594-, conjugated anti-

mouse or anti-rabbit IgG (1:600 each, Invitrogen, Carlsbad, CA). This was followed by total nuclei staining using Hoechst 33342 as described above.

**Flow Cytometry**

Prior to staining, non-specific Fc-mediated interactions were blocked by incubation with anti-mouse CD16/CD32. Cells were then labeled for microglia-specific marker allophycocyanin (APC)-conjugated CD11b. Cells were examined by flow cytometry using a BD LSR II instrument with BD FACS Diva 6.2.1 software (BD Biosciences, San Jose, CA) and analyzed for the amount of $CD11b^+$ microglia, the percentage of $CD11b^+$ cells that phagocytosed Fitc-tagged $A\beta_{42}$, and the amount of Fitc-tagged $A\beta_{42}$ phagocytosed as indicated by fluorescent intensity of Fitc in $CD11b^+$ cells with FlowJo software (FlowJo LLC, Ashland, OR).

**RT-PCR**

All primers listed below in **Table S2** were ordered from Invitrogen (Carlsbad, CA). Reverse transcription of RNA and RT-PCR was performed in a Rotor-Gene Q cycler (Qiagen, Valencia, CA). cDNA conversion from 50 ng mRNA was performed using the QuantiTect Reverse Transcription kit from Qiagen (Valencia, CA) followed by RT-PCR with Rotor-Gene SYBR Green PCR mix (Qiagen, Valencia, CA) and 1 μM of both forward and reverse primer pairs for the following genes: CD200R1, CD200R3, Trem2, Tyrobp, TGF-β1, TGF-βR1, IGF-1, BDNF, NT3, GDNF, and GAPDH. For analysis of CD200R1, CD200R3, Trem2, and Tyrobp, the following cycle was performed: initial activation at 95°C for 5 minutes, followed by 40 amplification cycles

by denaturation at 95°C for 5 seconds to annealing and extension at 60°C for 10 seconds. For analysis of TGF-β1, TGF-βR1, IGF-1, BDNF, NT3, and GDNF, the following cycle was performed: initial activation at 95°C for 5 minutes, followed by amplification with 55 cycles of 95°C for 15 seconds to 60°C for 30 seconds to 72°C for 45 seconds. Gene expression was compared relative to expression in untreated cells using the comparative $C_T$ method ($\Delta\Delta C_T$ method).

**Immunoblotting**

Immunoblotting was performed using 20-30 μg protein on 16% SDS-polyacrylamide Tris-Tricine gels (Schagger, 2006). Electroblotting was performed on 0.45 μm-pore size PVDF membranes (Immobilon-P, Millipore, Billerica, MA). Membranes were blocked in 5% milk with Tris-Buffered Saline-Tween 20, and incubated with CD200 monoclonal Ab (1:200, rat, BD Biosciences, San Jose, CA) or anti-GFP polyclonal Ab (1:1000, rabbit, Abcam, Cambridge, MA). This was followed by incubation with HRP-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) or biotinylated anti-rat IgG plus HRP-conjugated streptavidin (Thermo Scientific, Rockford, IL). Immunoreactive bands were detected with SuperSignal West Pico Chemiluminescent substrate (Thermo Scientific, Rockford, IL).

**Table S1. Primary Antibodies Used**

| Antigen | Abbreviation | Dilution | Host | Company |
|---|---|---|---|---|
| cluster of differentiation 200 | CD200 | 1:100 | rat | BD Biosciences |
| bromodeoxyuridine | BrdU | 1:250 | rat | Abcam |
| doublecortin | Dcx | 1:500 | goat | Santa Cruz |
| nitrogen oxygen synthase-2 | NOS2 | 1:200 | rabbit | Santa Cruz |
| chitinase 3-like-3 | YM1 | 1:1000 | rabbit | Stem Cell |
| microglial marker | F4/80 | 1:1000 | rat | eBioscience |
| total Aβ | pan-Aβ | 1:200 | rabbit | Zymed |
| β-III tubulin | β3T | 1:500 | mouse | Promega |
| glial fibrillary acidic protein | GFAP | 1:500 | rabbit | Dako |
| allophycocyanin-tagged-cluster of differentiation antigen-11b | APC-CD11b | 1 μg/ml | mouse | eBioscience |
| P2Y12 purinergic receptor (resident microglia) | P2ry12 | 1:500 | rabbit | kindly provided by Dr. Oleg Butovsky |
| Sialoadhesin (peripheral monocyte) | CD169 | 1:100 | rat | AbD Serotec |
| ionized calcium binding adaptor molecule 1 | Iba1 | 1:500, 1:100 | rabbit, goat | Wako, Abcam |

**Table S2. Primer and Primer Sequences**

| Gene Target | Primer Sequence | Product Length | Reference |
|---|---|---|---|
| CD200R1 | F-GGAAAACCAGAAAACCGAAATG-3' | 70 | NM_021325.3 |
| | R-CCCCCATATTAAGAGCACTGCTA-3' | | |
| CD200R3 | F-AGTGCCACAGGGAGAAAAACA-3' | 69 | NM_029018, (Masocha, 2010) |
| | R-TCCAGTTATCAGTCAAATGGGAGAT-3' | | |
| Trem2 | F-GCCTTCCTGAAGAAGCGGAA-3' | 73 | NM_031254.3, (Hickman et al., 2013) |
| | R-GAGTGATGGTGACGGTTCCA-3' | | |
| Tyrobp | F-AGCCCTCCTGGTGCCTTCT-3' | 68 | NM_011662.2, (Kiialainen et al., 2005) |
| | R-GGCCTGTACGGGACTTAATC-3' | | |
| TGF-β1 | F-AGAGGTCACCCGCGTGCTAA-3' | 108 | NM_011577.1 |
| | R-TCCCGAATGTCTGACGTATTG-3' | | |
| TGF-βR1 | F-TGCCATAACCGCACTGTCA-3' | 72 | NM_009370.2, (Li et al., 2011) |
| | R-AATGAAAGGGCGATCTAGTGATG-3' | | |
| BDNF | F-ATGGGACTCTGGAGAGCCTGAA-3' | 552 | NM_001285416.1, (Huang et al., 2015) |
| | R-CGCCAGCCAATTCTCTTTTTGC-3' | | |
| GDNF | F-AAGCGCCCGCTGAAGACCAC-3' | 173 | NM_010275.3 |
| | R-AGGAAGCGCTGCCGCTTGTT-3' | | |
| IGF-1 | F-ATCTGCCTCTGTGACTTCTGA-3' | 176 | NM_010512.4 |
| | R-GCCTGTGGGCTTGTTGAAGT-3' | | |
| NT3 | F-CCGTCAGCCAGGATAATGATGAGATCT-3' | 77 | NM_008742.3 |
| | R-GCTGTTGCCTTGGATGCCACG-3' | | |
| GAPDH | F-ACGGCCGCATCTTCTTGTGCA-3' | 71 | NM_008084.3 |
| | R-CCAGGCGCCCAATACGGCC-3' | | |

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