Supporting Information

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SI Methods

Animals. Scavenger receptor class B type I (*SR-BI*) heterozygous deficient mice were obtained from The Jackson Laboratory, were on C57Bl6/J;129S2 mixed genetic background, and had been backcrossed for at least six generations to C57Bl6/J background. Transgenic mice overexpressing a human amyloid precursor protein (APP) transgene under the PDGF promoter, containing the Swedish (K670M/N671L) and Indiana (V717F) mutations referred to as J20, were kindly provided by Dr. L. Mucke (Gladstone Institutes, University of California, San Francisco, CA) and were on C57Bl6/J genetic background. All *SR-BI* heterozygous and homozygous mice used in experiments for apolipoprotein E (ApoE), ApoAI, and CD206 expression in the brain were 9 mo old. *SR-BI*$^{+/+}$ littermate mice were used as positive controls. All mice analyzed for amyloid-β (Aβ)-related pathology were 11 mo old. Only male mice were analyzed for behavioral and memory deficits; in all other experiments, mice of both genders were used. Male 11-mo-old C57Bl6/J mice were used as controls in the behavioral tests. All mice were bred at the specific pathogen free Transgenic Mouse Facility of the Biomedical Research Institute of the Academy of Athens and maintained on a standard chow diet containing 5% fat (Teklad; Harlan).

Protein Extraction from Brain Tissue. Protein extraction from brains of wild-type, *SR-BI*$^{+/-}$, *SR-BI*$^{-/-}$, J20, and J20/*SR-BI*$^{+/-}$ mice was done in consecutive steps. Tissues were homogenized in five volumes of ice-cold suspension buffer (0.1 M NaCl, 0.01 M Tris·HCl, 0.001 M EDTA, pH 7.6) containing complete protease inhibitor (Roche Applied Science) in a Tissuemite homogenizer (Wheaton). The homogenate was centrifuged at 4 °C for 15 min at 12,500 rpm in a microcentrifuge (Eppendorf). The supernatant (soluble fraction) was removed and used to evaluate soluble ApoE, ApoAI, CD206, and superoxide dismutase-2 (SOD2). In the case of the J20 and J20/*SR-BI*$^{+/-}$ mice, the pellets from the previous step were then resuspended in ice-cold lysis buffer [containing 10% glycerol, 1% Triton X-100, and complete protease inhibitor (Roche Applied Science) in phosphate buffered saline (PBS)] and centrifuged at 4 °C for 10 min at 9,000 rpm. The supernatant (lysis fraction) was removed and used to evaluate soluble Aβ (ELISA) and ApoE and SR-BI. The pellet from the lysis step (insoluble fraction) was finally solubilized in 5 M guanidine hydrochloride in 50 mM Tris·HCl (pH 8.0) at room temperature for about 3 h with continuous rotation to evaluate plaque-associated ApoE and Aβ. Brain tissue from all animals was extracted in an identical manner, and all fractions were immediately frozen at −80 °C until analysis.

Western Analysis. Protein concentration in brain tissue was determined by BCA Protein Assay (Pierce) before analysis. Equal amounts of total protein were resolved on SDS/PAGE, electrophoretically transferred to polyvinylidene difluoride (Millipore) or nitrocellulose membranes (Schleicher and Schuell), and immunoblotted using either an anti–SR-BI (1/1000; Novus Biologicals), anti-ApoE (M20; 1:2,000; Santa Cruz Biotechnology), anti-ApoAI (1:200; Santa Cruz Biotechnology), anti-CD206 (1:300; Serotec), anti-CD163 (1:300; Serotec), anti-APP (6E10; 1:2,000; Sigma), anti-SOD (1:500; BD Biosciences), anti-GAPDH (1:1,000; Santa Cruz Biotechnology), or anti-tubulin (1:500; Sigma) antibody. Blots were developed using enhanced chemiluminescence (Amersham Biosciences) according to the manufacturer's recommendations. Densitometric analysis was performed using National Institutes of Health ImageJ software.

Histological Analysis. Fixed hemibrains of 11-mo-old mice were cut in 40-μm sagittal floating sections from the genu of the corpus callosum to the most caudal hippocampus by using a vibratome (Leica). Six sections, 240 μm apart and spanning the entire length of the hippocampus, were chosen for staining and subsequent analysis. For thioflavine-S staining, sections were immersed for 9 min in 1% thioflavine-S aqueous solution (Sigma), followed by washing and dehydration in 80% ethanol for 3 min (twice) and in 95% ethanol for 3 min, followed by three washes in ddH_2O. Sections were mounted on Superfrost Plus slides (Fisher) and coated with Vectashield aqueous mounting medium for fluorescence (Vector Laboratories). Briefly, for Aβ immunohistochemistry, after washing in PBS, sections were incubated in 88% formic acid for 5 min, incubated for 30 min with 0.3% H_2O_2 in PBS-T (0.01 M PBS, pH 7.4, 0.1% Triton-X-100), transferred to blocking solution containing 15% normal horse serum (Vector Laboratories) in PBS-T for 60 min, and incubated overnight at 4 °C in blocking solution containing the monoclonal biotinylated 6E10 primary antibody (1:1,000; Signet) to detect Aβ deposits. Sections were then washed with PBS-T, and incubation in avidin-biotinylated horseradish peroxidase complex (ABC Elite; Vector Laboratories) followed for 45 min at room temperature. Peroxidase labeling was visualized by using 3,3-diaminobenzidine/nickel (Vector Laboratories). After a 1-min incubation, sections were washed, mounted on glass slides, dehydrated in increasing ethanol concentrations from 50 to 100% followed by xylene, and coverslipped with dibutyl phthalate xylene mounting medium (VWR). The specificity of immunoreactivity was confirmed by the lack of signal when applying the same protocol on brain sections of wild-type littermates. Images were analyzed with National Institutes of Health ImageJ software. The thioflavine-S and the Aβ load were defined as the percent area covered by thioflavine-S (% thioflavine-S load) and Aβ immunoreactivity (% Aβ load), respectively. Vascular thioflavine-S load was determined by subtracting the percent area of cortex and hippocampus stained by thioflavine-S that includes fibrillar amyloid plaque deposits and excludes fibrillar vascular amyloid deposits from the percent area stained by thioflavine-S that includes both fibrillar amyloid plaques and fibrillar vascular amyloid deposits. Imaging for immunofluorescence was performed on a Leica DMRA 2 microscope (magnification 4×) and for Aβ immunohistochemistry on a Leica DMLS 2 microscope (magnification 2.5×), and image capture was performed using Leica Application Suite (version 2.8.1) software.

Double Labeling and Fluorescent Immunohistochemistry. For CD206 labeling, 10-μm acetone-fixed cryostat sections were blocked for 2–3 h in 10% FCS, 1% BSA, 0.01% Triton X-100 in PBS and then incubated overnight at 4 °C with anti-CD206 (1:300; Serotec) in 1% FCS, 1% BSA, 0.01% Triton X-100 in PBS. Sections were washed the next day with PBS and developed with an anti-rat antibody (1:200; Oxford Biotechnology). For CD206 plus SR-BI double labeling, 10-μm acetone-fixed cryostat sections were blocked for 20 min in PBS supplemented with 20% goat serum, incubated with anti-CD206 (1:100; Serotec) and anti–SR-BI (1:250; Novus Biologicals) overnight at 4 °C, and diluted in PBS supplemented with 3% goat serum. After incubation with the primary antibody, sections were washed three times in PBS, incubated with an anti-rat secondary antibody (1:200; Oxford Biotechnology) and an anti-rabbit antibody (1:300; The Jackson Laboratory) for 1 h at room temperature, and washed again in PBS. For thioflavine-S plus Iba-1 labeling, 40-μm sagittal floating

sections were blocked for 2–3 h in 10% FCS, 1% BSA, 0.01% Triton X-100 in PBS and then incubated overnight at 4 °C with anti–Iba-1 (1:250; Wako) in 1% FCS, 1% BSA, 0.01% Triton X-100 in PBS. Sections were washed the next day with PBS and developed with an anti-rabbit antibody (1:300; The Jackson Laboratory) and then processed for thioflavine-S staining. They were treated with 1% thioflavine-S for 5 min, differentiated twice in 70% EtOH, and washed in PBS solution. For thioflavine-S plus glial fibrillary acidic protein (GFAP) labeling, 40-μm sagittal floating sections were blocked for 1 h in tris buffered saline (TBS), 0.4% Triton X-100, supplemented with 5% goat serum, and then incubated for 1 h with anti-GFAP (1:1,000; Sigma) in the blocking solution. Sections were then washed with TBS and developed with a goat anti-rabbit antibody (1:300; The Jackson Laboratory), all at room temperature, and then processed for thioflavine-S staining as mentioned above. For thioflavine-S plus SR-BI labeling, 10-μm acetone-fixed cryostat sections were incubated with anti–SR-BI (1:250) as mentioned above, exposed to anti-rabbit secondary antibody (1:300; The Jackson Laboratory), and then processed for thioflavine-S staining. They were treated with 1% thioflavine-S for 5 min, differentiated twice in 70% EtOH, and washed in PBS solution. Finally, for SR-BI and Aβ double labeling, 10-μm acetone-fixed cryostat sections were processed as for GFAP labeling with primary and secondary antibody dilutions as follows: anti–SR-BI (1:250; Santa Cruz Biotechnology), anti-Aβ (1:500; Sigma), anti-rabbit (1:300; The Jackson Laboratory), and anti-mouse FITC antibody (1:500; Sigma). Floating sections were mounted on slides and all slides were coverslipped with Vectashield aqueous mounting medium for fluorescence (Vector Laboratories). Fluorescent photomicrographs were captured using a Leica DMRA 2 microscope or a Zeiss confocal microscope and exported to Leica Application Suite (version 2.8.1) software.

Cell Isolation and Culture. We isolated cortical microglia from neonatal (1- to 2-d-old) *SR-BI* heterozygote or wild-type C57BL/6 mice according to previously studied methods (1–3). Briefly, we isolated brains under sterile conditions and incubated cerebral cortices in trypsin-EDTA for 45 min at 37 °C. We then added full DMEM (supplemented with 10% FCS, 1 mM penicillin-streptomycin) and dissociated the brains by trituration. Subsequently, we plated cerebral cortex material in 25 cm^2 flasks (Greiner Bio-One) and changed the media every 2–3 d. Fourteen days after plating, we isolated microglia by shaking in an incubator-shaker at 200 rpm for 2 h at 37 °C. We isolated peripheral macrophages (MΦ) from adult *SR-BI* heterozygote or wild-type C57BL/6 mice according to standard immunological methods by intraperitoneally injecting mice with 1,000 μL of 3% (wt/vol) sterile thioglycollate solution diluted in PBS. Four days later, we i.p. injected mice with 10 mL of ice-cold PBS for peritoneal lavage. Then the peripheral MΦ were plated with complete DMEM supplemented with 10% FCS, 1 mM penicillin-streptomycin. After 30 min we changed the medium and allowed them to rest overnight. The following morning, we rinsed MΦ four times in ambient-temperature PBS and added fresh medium.

Aβ Phagocytosis Assay. We plated peripheral MΦ from *SR-BI* heterozygote or wild-type C57BL/6 mice on glass coverslips in 24-well culture plates (TPP) at 1×10^5 cells per well in complete DMEM as described above. We resuspended human synthetic $A\beta_{1–42}$ conjugated with Hilyte Fluor 488 ($A\beta_{488}$; AnaSpec) as suggested by the manufacturer and preaggregated it for 24 h at 37 °C. We added $A\beta_{488}$ at 1 μg/mL to MΦ cultures and pulsed cells for 4 h at 37 °C. We then rinsed cells three times in ambient-temperature PBS, and chased them for 15 min to allow $A\beta_{488}$ to concentrate into phagolysosomes. After an additional two rinses in complete DMEM and then two final rinses in PBS, we mounted coverslips in fluorescent mounting medium for confocal microscopy. We acquired three random 10× magnification fields and reported data two ways: as an $A\beta_{488}$-labeled area or number of MΦ/field containing $A\beta_{488}$. We performed the same procedure with microglia cultures from neonatal (1- to 2-d-old) *SR-BI* heterozygote or wild-type C57BL/6 mice.

Behavioral Studies. Mice were tested in three behavioral tasks in the following order: open field, novel object recognition, and water maze.

Open-field exploration. Mice were placed in a new field and their locomotor activity was recorded across two consecutive daily sessions. The floor of the field (40 × 40 × 40 cm) was marked out in nine compartments of equal size (three rows of three). Mice were placed in the corner of one compartment facing the wall, and their behavior was recorded for 5 min each day. The number of entries (four-paw criterion) in each compartment (crosses) was used as an indication of horizontal movement, and the number of rears as an indication of vertical movement. On the first day of testing, animals were considered to be in a novel environment, whereas on the second day in a more familiar one. The effect of genotype on the number of cross-overs or on the number of rears was assessed using a one-way ANOVA per genotype. Significance was defined as $P < 0.05$. All statistical analyses were performed using SPSS software (release 10.0.1).

Novel object recognition. Mice were tested in the novel object recognition task 1 d after the open field. The protocol we followed consisted of a familiarization session (three 5-min trials) and a probe trial. During familiarization, mice were placed at the center of the field and left to explore two identical objects made of Lego blocks. During the probe trial, which occurred 15 min after the last familiarization trial, mice were placed back into the same field but one of the objects was replaced by a novel Lego block object (different shape and color). Time spent observing each object (exploration time) was scored and the discrimination ratio [(time spent observing novel object − time observing familiar object) / (time spent observing novel object + mean time observing familiar object)] was calculated. The effect of genotype on the above parameter was assessed using a one-way ANOVA per genotype. Significance was defined as $P < 0.05$. All statistical analyses were performed using SPSS software (release 10.0.1).

Water maze. Three days after the novel object recognition task, mice were tested in the water maze. We used a circular pool (140 cm in diameter) situated in a room with discrete extramaze cues and filled with water that was rendered opaque with milk. A transparent movable escape platform (12 × 12 cm) was submerged 1 cm below the water surface. The protocol we followed consisted of a visible session, a training session, and a probe trial. During the visible session, mice were trained for three consecutive days to find a randomly located platform marked with a black flag. There were four 60-s trials per day (separated by 15-min intervals), each with a different starting position along the perimeter of the pool. At the end of each trial, mice were allowed on the platform for 20 s. In the training session, mice were trained to find a hidden platform (in a fixed position relative to visible extramaze cues) across four consecutive daily sessions. Each daily session consisted of four 60-s trials with four different starting positions (intertrial interval 15 min). At the end of each trial, mice were left on the platform for 20 s. One hour after the last training trial (on the fourth training day), animals were given a probe trial in the absence of the platform (60-s duration, starting position opposite the target quadrant, i.e., the quadrant in which the platform was located during training). The behavior of the animals was recorded and analyzed using EthoVision software (Noldus). During visible and training sessions, we analyzed latency (time in seconds to reach the platform) as a parameter of learning, as well as swim speed to ensure that behavior in the water maze does not reflect changes in activity. The values obtained were averaged per mouse within each daily session. The effect of genotype on these parameters was assessed using a one-

way ANOVA with repeated measures (day). In the probe trial, we analyzed the time spent in the target and opposite quadrants. The effect of genotype on this parameter was assessed using a two-way ANOVA with quadrant and genotype as independent factors. A value higher than 20 s (>33% of probe trial duration) in the target quadrant was interpreted as a spatial bias. Significance was defined as $P < 0.05$. All statistical analyses were performed using SPSS software (release 10.0.1).

Statistical Analysis. Two-tailed unpaired Student's *t* tests and ANOVA were used for statistical analysis. All statistical analyses were performed using Prism (version 4.0; Graphpad Software).

1. Brionne TC, Tesseur I, Masliah E, Wyss-Coray T (2003) Loss of TGF-β1 leads to increased neuronal cell death and microgliosis in mouse brain. *Neuron* 40:1133–1145.
2. Wyss-Coray T, Lin C, Sanan DA, Mucke L, Masliah E (2000) Chronic overproduction of transforming growth factor-β1 by astrocytes promotes Alzheimer's disease-like microvascular degeneration in transgenic mice. *Am J Pathol* 156:139–150.
3. Town T, et al. (2008) Blocking TGF-β-Smad2/3 innate immune signaling mitigates Alzheimer-like pathology. *Nat Med* 14:681–687.

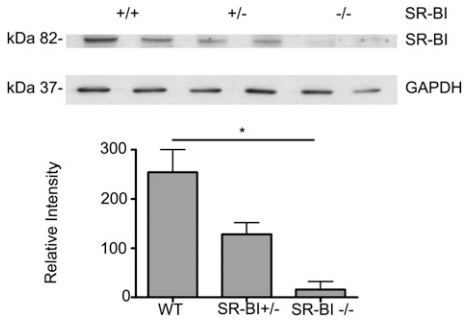

Fig. S1. *SR-BI*$^{+/-}$ mice have reduced SR-BI protein levels in the brain. Western and densitometric analysis of brain lysates of *SR-BI*$^{+/+}$, $^{+/-}$, and $^{-/-}$ mice showed a significant decrease in SR-BI (*$P < 0.05$).

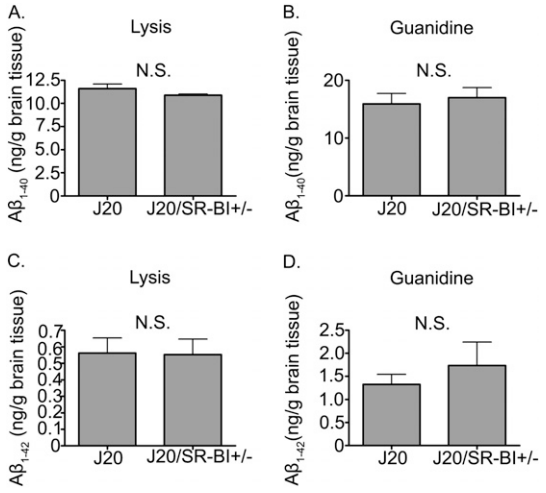

Fig. S2. Quantitation of $A\beta_{1–40}$ and $A\beta_{1–42}$ levels by ELISA showed a small but nonsignificant (N.S.) increase in $A\beta_{1–42}$ levels. (*A*) $A\beta_{1–40}$ levels in brain lysis fraction of J20 and J20/*SR-BI*$^{+/-}$ mice. (*B*) $A\beta_{1–40}$ levels in brain guanidine fraction of J20 and J20/*SR-BI*$^{+/-}$ mice. (*C*) $A\beta_{1–42}$ levels in brain lysis fraction of J20 and J20/*SR-BI*$^{+/-}$ mice. (*D*) $A\beta_{1–42}$ levels in brain guanidine fraction of J20 and J20/*SR-BI*$^{+/-}$ mice.

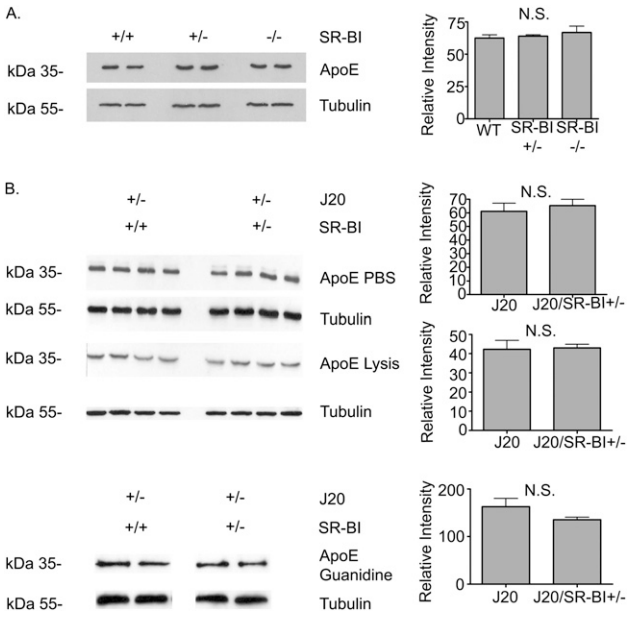

Fig. S3. ApoE levels are not affected by SR-BI in the mouse brain. (*A*) PBS-extractable ApoE levels were determined by Western blotting in brain lysates of *SR-BI*$^{+/+}$, $^{+/-}$, and $^{-/-}$ mice. Western blots show two representative samples per group. (*B*) ApoE levels were determined by Western blotting in the soluble (PBS), lysis, and insoluble fractions of J20 and J20/*SR-BI*$^{+/-}$ brains. Western blots show two to four representative samples per group. Protein levels were standardized with tubulin. Densitometric analysis showed no differences.

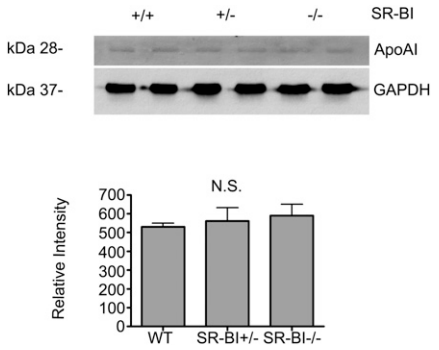

Fig. S4. ApoAI levels are not affected by SR-BI in the mouse brain. ApoAI levels were determined by Western blotting in brain lysates of *SR-BI*$^{+/+}$, $^{+/-}$, and $^{-/-}$ mice. Western blotting shows two representative samples per group. Protein levels were standardized with tubulin. Densitometric analysis showed no differences.

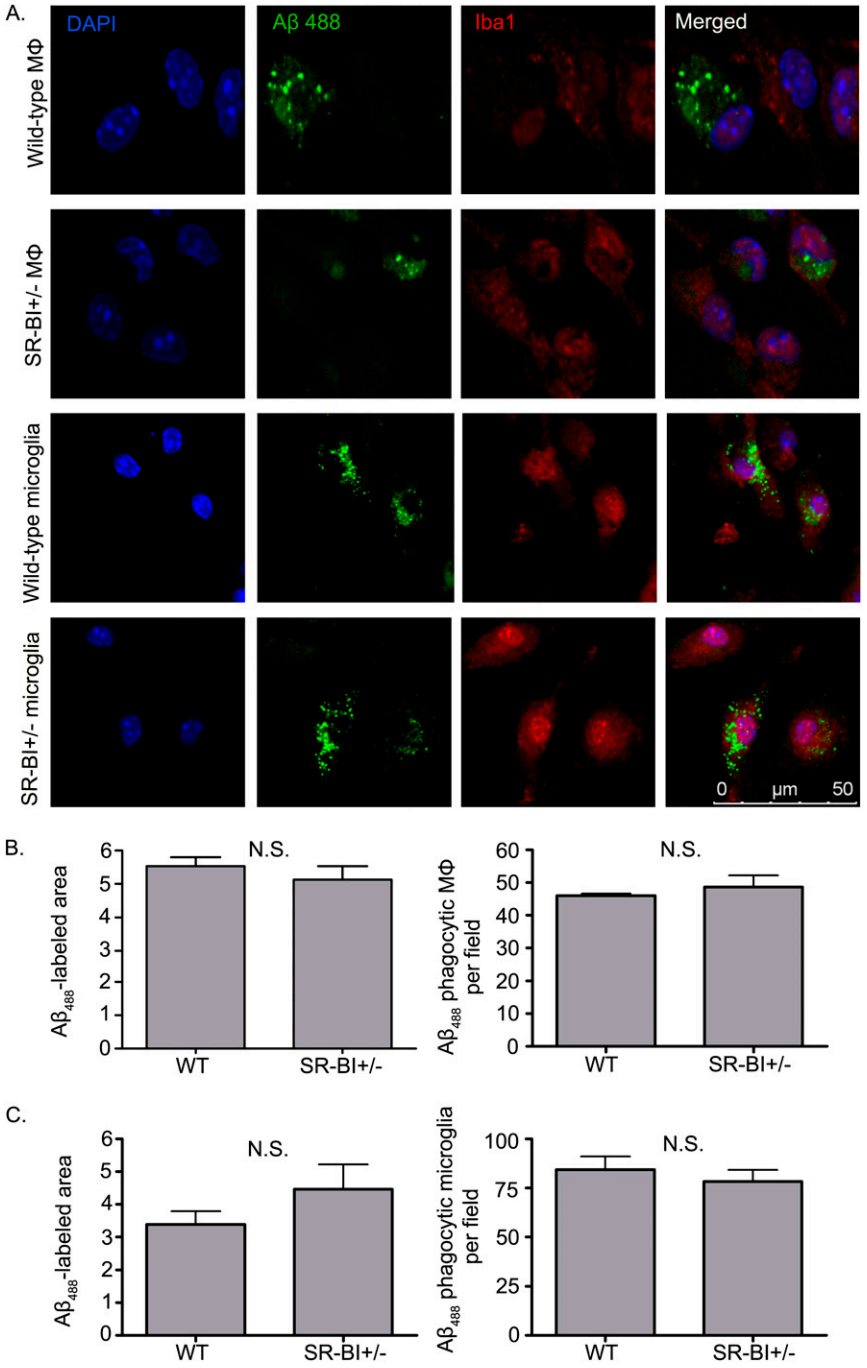

Fig. S5. Peripheral macrophages and microglial cultures from wild-type and *SR-BI*$^{+/-}$ mice showed no differences in Aβ phagocytic activity. (*A*) Confocal microscope images of primary macrophages and microglia stained with Iba-1 showing phagocytosed labeled Aβ. (Scale bar, 50 μm.) (*B*) Quantification of Aβ-labeled area on macrophage confocal images (n = 3 randomly selected fields per group) showed no difference (*Left*). Numbers of Aβ phagocytic cells per field showed no difference between wild-type and *SR-BI*$^{+/-}$ peripheral macrophages (*Right*). (*C*) Quantification of Aβ-labeled area on microglia confocal images (n = 3 randomly selected fields per group) showed no difference (*Left*). Numbers of Aβ phagocytic cells per field showed no difference between wild-type and *SR-BI*$^{+/-}$ microglia (*Right*).

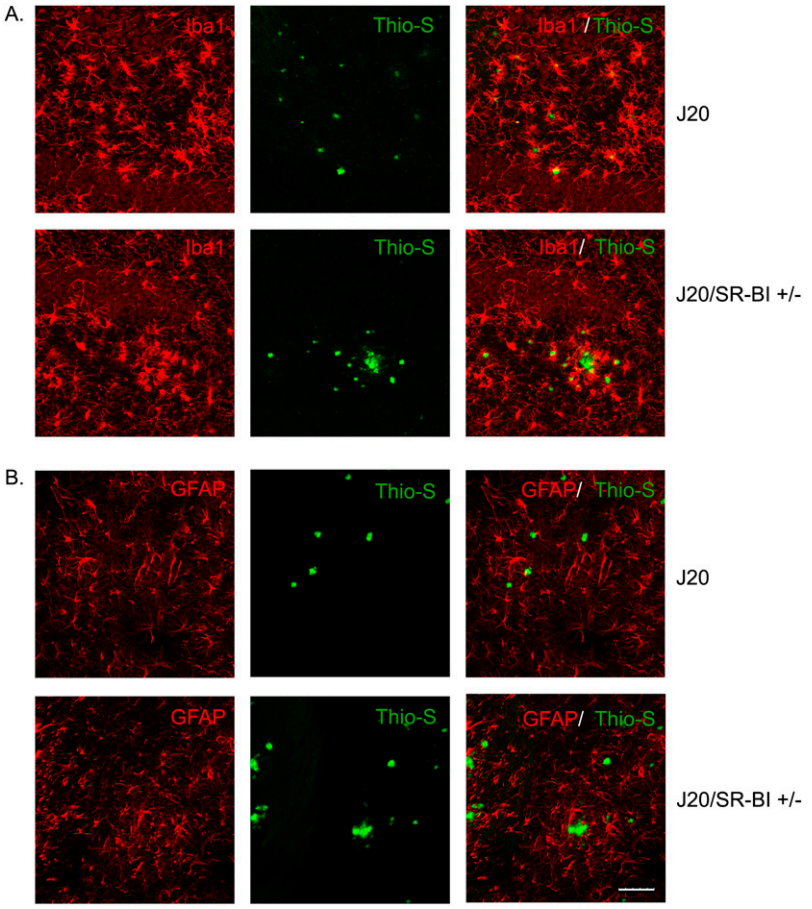

Fig. S6. J20 and J20/*SR-BI*$^{+/-}$ mice develop a similar astrocytic and microglial response. (*A*) Iba-1 immunostaining for activated microglia and thioflavine-S staining for amyloid plaques in J20 and J20/SR-BI$^{+/-}$ mouse brains. (*B*) GFAP immunostaining for activated astrocytes and thioflavine-S in J20 and J20/*SR-BI*$^{+/-}$ mouse brains. (Scale bar, 250 μm.)

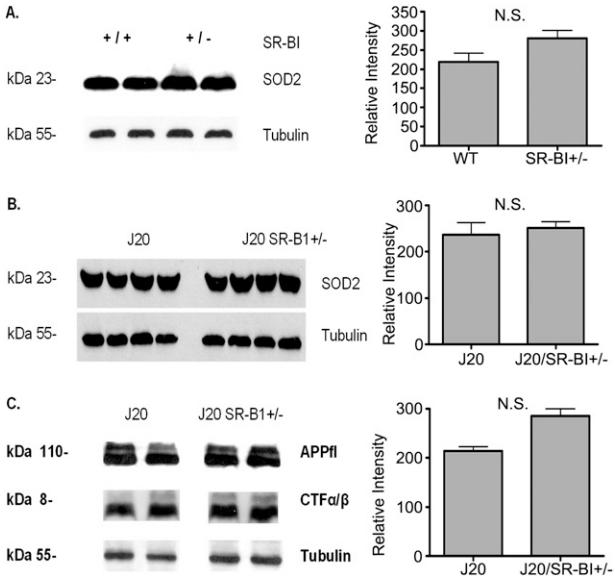

Fig. S7. Oxidative stress and APP processing are not affected in J20 and J20/*SR-BI*$^{+/-}$ mice. (*A*) SOD2 was not affected by SR-BI reduction or deletion in *SR-BI*$^{+/+}$, $^{+/-}$, or $^{-/-}$ mice. Whole-brain lysates from *SR-BI*$^{+/-}$, $^{-/-}$, and control mice were analyzed by immunoblotting and densitometry. Protein levels were standardized with tubulin. (*B*) Similarly, SOD2 was not changed in J20 and J20/*SR-BI*$^{+/-}$. Whole-brain lysates from J20 and J20/*SR-BI*$^{+/-}$ mice were analyzed by immunoblotting and densitometry. Protein levels were standardized with tubulin. (*C*) Same levels of APP full-length (APPfl) and carboxy-terminal fragments (CTFs) in J20 and J20/*SR-BI*$^{+/-}$ mice. Whole-brain lysates from J20 and J20/*SR-BI*$^{+/-}$ mice were analyzed by immunoblotting and densitometry. Protein levels were standardized with tubulin.