Supporting Information

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SI Materials and Methods

Experimental Animals. Mice, including WT C57BL/6J mice and mice deficient in gp91^{phox} (gp91$^{phox-/-}$) or CD11b (CD11b$^{-/-}$), were purchased from The Jackson Laboratory. All animals were housed in a pathogen-free facility at the National Institute of Environmental Health Sciences (NIEHS). Animal housing, breeding, and experiments were approved by NIEHS and performed in strict accordance with National Institutes of Health guidelines.

Primary Neuron-Enriched Cultures. The cultures were established as described previously (1) with minor modifications. In brief, mesencephalic tissues were collected from mouse or rat E14 embryos, and the cells were seeded on 6- or 24-well plates or MakTek dishes coated with poly-D-lysine (Sigma-Aldrich). Two days later, 10 μM arabinofuranosyl cytidine (Ara-C; Sigma-Aldrich) was added to the cultures to remove the glia and enrich the neurons. On day 6 after cell seeding, Ara-C was removed using fresh media. Cultures established by this method contain neurons with >95% purity. In the experiments in which α-syn was overexpressed or knocked down, the enriched neuronal cultures were infected on day 6 with an AAV2 or lentiviral vector and incubated overnight, followed by maintenance in fresh media. On day 14, these neuron-enriched cultures, either intact or infected, were used for various experimental purposes. In our pilot study, in which GFP carried by an AAV2 vector was used as an expression reporter, we found that the fluorescent signal normally appeared on day 11 (4 d after infection), and a stronger signal was detected by day 14 (7 d after infection), suggesting successful infection and continued cell viability.

Although the infected cells remained viable on day 14, they appeared dysmorphic, indicating an "unhealthy" status. Cultured in parallel, uninfected (intact) neuron-enriched cultures exhibited a similar morphology (Fig. S1*F*), suggesting that such an unhealthy phenotype may possibly result from the lack of glial contacts, rather than from infection with the viral vector.

Cultures of Mixed Glia and Harvesting of Microglia. The brains of mouse or rat pups were dissected on postnatal day 1 as described previously (1), and the cells were seeded on poly-D-lysine–coated T175 flasks. Two weeks later, the microglia were harvested by shaking the flasks at 180 rpm for 30 min by using an Excella E24 shaker (New Brunswick Scientific, Edison, NJ). More than 98% of the collected cells were microglia with CD11b-positive immunostaining. These microglia were either used for immediate experiments, such as chemotaxis and adhesion assays, or seeded onto 6- or 96-well plates overnight for assays of protein phosphorylation or H_2O_2 generation.

AAV2 Vectors and Infection of Rat Primary Neurons. AAV2 vectors harboring the reporter gene GFP or human α-syn driven by the chicken beta-actin (CBA) promoter were gifts from the Michael J. Fox Foundation. To infect rat primary neuron-enriched cultures cultured in 6- or 24-well plates or MakTek dishes, 10 μL (for each well in the 6-well plates or MakTek dishes) or 2.5 μL (for each well in the 24-well plates) of the vectors (1.0×10^{13} Vg/mL) was added overnight on day 6 after cell seeding, and the cultures were maintained in fresh media for 7 d, when the expression of GFP or α-syn was verified via microscopy or Western blot analysis.

Retroviral Packing of Lentiviral Vectors and Infection of Rat Primary Neurons. Retroviral packing and infection were performed as described previously (2). In brief, human 293FT cells (Invitrogen) were placed on poly-D-lysine–precoated dishes and transfected with MegaTran1.0 transfection reagent containing the packing plasmids and the pGFP-C-shLenti vector carrying scrambled or rat α-syn–specific shRNA (Origene Technologies). On days 2 and 3 after transfection, the culture media were collected to obtain the viral vectors. To infect rat neuron-enriched cultures that had been established for 6 d, the media collected from the transfected 293FT cell cultures were added overnight to each culture, along with 6 μg/mL polybrene (Fisher Scientific). After 48 h, the expression of α-syn was evaluated via fluorescence microscopy and Western blot analysis. In some experiments, the infected cultures were further maintained in fresh media for 7 d, when the cells exhibited an unhealthy morphology, and were used for various experimental purposes.

Knockdown of Lyn in HAPI Cells Using SiRNA. HAPI cells, a rat microglia-derived cell line (3), were seeded onto six-well plates. The next day, when the cultures became 40–50% confluent, the cells were transfected by adding Opti-MEM reduced serum media containing a mixture of Lipofectamine RNAiMAX transfection reagent (7.5 μL per well; Life Technologies) and ON-TARGETplus SMARTpool rat Lyn-specific siRNA (40–80 pmol per well; GE Dharmacon) for 6 h and were subsequently maintained in fresh DMEM media supplemented with 10 ml/100 ml FBS. On days 3 and 10 after transfection, the cells were harvested to evaluate protein expression or were used for various experiments.

Preparation of Recombinant Human α-Syn. Recombinant human α-syn (endotoxin level, <0.024 EU/μg; rPeptide) was dissolved in water (1 mg/mL ~ 72 μM) and incubated with agitation at 37 °C for 7 d. As described previously (1), this process allowed α-syn to form the oligomers used in this study.

Isolation of Rat Peripheral Blood Monocytes. Rat monocytes were purified from the peripheral blood as described previously (4). In brief, blood was collected from male rats (8–12 wk old) by cardiopuncture into BD PosiFlush Heparin Lock Flush Syringes. After centrifugation at 400 × *g* for 5 min, the plasma-rich supernatant was removed, and the blood cells were diluted in an equal volume of PBS. Next, PBS containing blood cells was gently loaded on the top of the Ficoll-Paque PREMIUM solution (GE Healthcare Life Sciences) and centrifuged at 400 × *g* for 30 min. The mononuclear leukocytes were harvested from the interface of PBS and Ficoll solution and washed in modified PBS containing 0.18% glucose, 0.25% BSA, and 10 mM Hepes. After seeding on poly-D-lysine–coated culture dishes at 37 °C for 1 h, nonadherent mononuclear leukocytes were removed by five washes, yielding monocyte preparations of >80% purity in the dishes.

Migration Assays Using 96-Well Boyden Chambers. In a 96-well Boyden chamber (Corning), each bottom well was filled with 200 μL of serum-free media containing 0.1% BSA, 1×10^{-7} M fMLP (EMD Chemicals), or 1.0 μM recombinant human oligomeric α-syn in the presence or absence of the control IgG or an anti–α-syn Ab (Abcam). Before the insert frame was placed back on the plate and incubated at 37 °C overnight, each pore-containing (5 μm diameter) filter insert was loaded with 1×10^5 microglia, HAPI cells, or rat monocytes pretreated with the isotype-control IgG or an anti-CD11b Ab (Biolegend) or a compound such as Apo (0.25 mM; EMD Chemicals), the Src inhibitor PP2 (10 μM; EMD Chemicals), Cyclosporin H (1.0 μM; Santa Cruz Biotechnology), or catalase (100 U/well; EMD Chemicals). The number

of transmigrated cells was measured using a CytoQuant Kit (Life Technologies) according to the manufacturer's protocol.

Migration Assays Using 24-Well Boyden Chambers. Enriched mouse or rat primary neurons were cultured in the bottom wells of 24-well Boyden chambers. In these cultures, <1 microglia per field was found under a 10× objective. A total of 1.0×10^5 microglia pretreated with the isotype-control IgG or an anti-CD11b Ab or a compound such as DMSO, Apo, PP2, or catalase were loaded on each insert, which contained pores (5 μm diameter) in its filter membrane. Then the inserts were placed back on the bottom chamber. In some experiments, the cultures in the bottom wells were treated with the control IgG or an anti–α-syn Ab (Abcam) when the migration assays were initiated. After overnight incubation, the cultures were stained with calcein-AM (Life Technologies) and Alexa Fluor 594-conjugated isolectin (Life Technologies). The isolectin-positive cells in 15 random fields per well were counted as microglia. Calcein-AM labels all live cells, and isolectin has been used as a marker of endothelial cells (5) or microglia (6). Here isolectin staining was specific to microglia, because there were no endothelial cells in our cultures.

Chemotaxis Assays Using Live Cell Imaging. Intact or viral vector-infected enriched rat primary neurons were cultured in 24-well plates, and assays for microglia or HAPI cells overlapping neurons were established. To this end, a total of 3.0×10^5 microglia pretreated with the isotype-control IgG or an anti-CD11b Ab or a compound such as DMSO, Apo, PP2, or catalase were loaded directly on each well in which intact or viral vector-infected enriched rat neurons were growing. Alternatively, 1.0×10^5 HAPI cells transfected with scrambled or Lyn siRNA or treated with DMSO or PP2 were loaded. On the next day, the cells were labeled with calcein-AM (cytoplasm of both neurons and microglia), Alexa Fluor 594-conjugated isolectin (microglia only), and Hoechst (cell nuclei). Fifteen fields in each well for each experimental condition were selected at random for imaging with an Axio Observer epi-fluorescent microscope (Carl Zeiss Microscopy) equipped with a 10× objective.

To quantify the amount of microglia overlapping neurons, neuronal regions were defined according to the sharply defined nuclear stain, which largely overlapped the less well-defined cell body stain in low-magnification imaging, using Imaris 7.7 (Bitplane). Microglia-specific isolectin staining within the neuronal regions of interest was then used to count the number of microglia. The software was set to automatically detect microglial cells, defining a single microglial cell as a red signal 5–10 μm in diameter; when multiple objects clustered together, the software automatically segmented them in terms of the best-fit mode and then counted the number of microglia.

Chemotaxis Assessment Using Under-Agarose Migration Assays. These assays were performed to examine the ability of the cells to migrate toward the source of H_2O_2 because wells made in agarose gels can hold H_2O_2, allowing it to slowly diffuse into the body of the gels, establishing a gradient throughout the wells. Following the procedure described previously (7, 8), the agarose solution was prepared and poured into 60-mm tissue culture dishes. When solidified, the gels were cut to generate five wells (3 mm diameter) in each dish using a template in which one hole located in the center was surrounded by four other holes at a distance of 2.2 mm from the central hole. The potential chemoattractants, H_2O_2 (50 μM), and their corresponding controls were added to the middle well, and 1.0×10^5 freshly isolated primary microglia or HAPI cells in serum-free media with or without the Src inhibitor PP2 and catalase were loaded in the peripheral wells. After overnight incubation, the gels were fixed and stained using a HEMA 3 Kit (Fisher Scientific). The number of chemotactic cells was determined by subtracting the number of cells migrating away from the chemoattractant from the number of cells migrating toward the chemoattractant.

Co-IP. To determine whether recombinant human α-syn binds to Myc/DDK-tagged CD11b (OriGene Technologies), an equimolar mixture of these two proteins was incubated in a cold room overnight and further incubated in anti-Myc–tagged magnetic beads (MBL International) in PBS at room temperature (RT) for 30 min. After multiple washes with PBS, the beads were boiled in Laemmli sample buffer (Bio-Rad), and the supernatant was subjected to SDS/PAGE. The proteins were transferred to PVDF membranes and detected using antibodies against α-syn (EMD Millipore) or DDK peptide (OriGene Technologies). In parallel experiments, α-syn or CD11b alone was incubated with the beads to serve as controls.

To evaluate the ability of recombinant human α-syn to bind to microglia-derived CD11b, α-syn was mixed with WT or $CD11b^{-/-}$ microglial lysates containing IP lysis buffer (Thermo Scientific) and protease inhibitor mixture (Thermo Scientific) at 4 °C overnight. Anti–α-syn Ab (Abcam) or control IgG was conjugated to the protein G magnetic beads using a Dynabeads Protein G Immunoprecipitation Kit (Life Technologies). Next, the mixtures of α-syn and microglial lysates were incubated in the Ab- or control IgG-conjugated protein G magnetic beads at RT for 30 min. After washing, the samples were eluted, followed by boiling in Laemmli sample buffer (Bio-Rad) and resolution via SDS/PAGE. The proteins were then transferred to PVDF membrane and immunoblotted using an anti-CD11b (Abcam) or α-syn Ab (EMD Millipore). The whole-cell lysates were immunoblotted using an anti–α-tubulin Ab (Abcam) as a protein loading control.

Flow Cytometry. After blocking with 0.5% BSA or 2.0 μM α-syn in the presence of 10 μg/mL rat anti-mouse CD16/CD32 Ab (BD Biosciences), WT mouse microglia were labeled with APC-conjugated to the anti-CD11b Ab (BioLegend). Alternatively, the WT or $CD11b^{-/-}$ mouse microglia were preincubated in an anti-CD11b blocking Ab or the control IgG (BioLegend) before staining with Dylight 488-conjugated α-syn (Dylight 488 protein labeling kit: Thermo Scientific). The APC and Dylight 488 fluorescent signals were analyzed using a LSR II flow cytometer (BD Biosciences). All procedures were performed on ice.

Adhesion Assays. The surfaces of 96-well plates were coated with 0.5% BSA or 2.0 μM α-syn. Calcein-AM–labeled WT or $CD11b^{-/-}$ microglia were preincubated in 10 μg/mL anti-CD11b Ab or the control IgG (BioLegend) on ice for 30 min, and then 1.0×10^5 cells were added to each well at 37 °C for 1 h. After three washes, the plates were scanned using a 96-well plate fluorescence reader.

Immunofluorescence. To examine the cell surface expression of CD11b, freshly isolated mouse microglia were preincubated in 0.1% BSA or 2.0 μM α-syn in the presence of 10 μg/mL rat anti-mouse CD16/CD32 Ab (BD Biosciences) for 30 min and then labeled with APC-conjugated anti-CD11b Ab on ice for 30 min. To demonstrate the membrane translocation of $p47^{phox}$ and cell spreading, mouse primary microglia or HAPI cells, either intact or pretreated with scrambled or Lyn-specific siRNA (OriGene Technologies), were seeded on laminin-coated coverslips overnight. The cells were stimulated using 0.1% BSA, 250 μM α-syn, or 10 μM H_2O_2 at 37 °C for 30 min and then fixed with 4 g/100 ml paraformaldehyde. After blocking with PBS containing 5 ml/100 ml goat serum and 0.3% Triton-X, the cells were immunostained for $p47^{phox}$ (EMD Millipore) or phosphorylated cortactin (phospho-Y466 Ab; Abcam), followed by staining for F-actin and with Hoechst (Life Technologies). The samples were imaged and

analyzed using a Zeiss LSM 510 confocal microscope (Carl Zeiss Microscopy).

Detection of Membrane Translocation of p47^{phox} via Western Blot. HAPI cells were stimulated using 0.1% BSA or 250 nM α-syn and then lysed in a hypotonic lysis buffer (1.0 mM Tris, 1.0 mM KCl, 1.0 mM EGTA, 1.0 mM EDTA, and 0.1 mM DTT) containing protease inhibitor mixture (Thermo Scientific) and 1 mM PMSF (Thermo Scientific). The membrane fraction was extracted from the cell lysates as described previously (9) with minor alterations. In brief, the cell lysates were centrifuged at 1,600 × *g* for 15 min, and the supernatant was collected for further centrifugation at 40,000 × *g* for 2 h. The high-speed centrifugation supernatant, representing the cytosolic fraction, was then harvested. Meanwhile, the pellets, containing the membrane fragments, were dissolved in 1% Nonidet P-40 hypotonic lysis buffer. Both the high-speed supernatant and the resuspended pellet were subjected to SDS/PAGE. The proteins were transferred to PVDF membranes and detected using anti-p47phox and anti-gp91phox Abs (Cell Signaling). The relative amount of each protein was quantified using the Quantity One program (Bio-Rad). The whole-cell lysates were immunoblotted for α-tubulin as a loading control.

O_2^- Production Assays. The release of O_2^- from mouse microglia was measured based on the SOD-inhibitable reduction of the tetrazolium salt WST-1 (10). In brief, WT, CD11b$^{-/-}$ or gp91$^{phox-/-}$ mouse primary microglia were seeded on 96-well plates overnight. After two washes with HBSS without phenol red, 50 μL of HBSS with or without SOD (50 U/mL) was added to each well, along with 50 μL of WST-1 (1 mM in HBSS) and 50 μL of HBSS containing the vehicle control, 100 nM PMA, or 250 nM α-syn. The absorbance at 450 nm was measured using a SpectraMax Plus microplate spectrophotometer (Molecular Devices) every 2 min for 30 min. The amount of O_2^- produced was determined according to the difference between the absorbance values in the presence and absence of SOD.

Extracellular and Intracellular H_2O_2 Assays. The level of extracellular H_2O_2, which was released from microglia into the culture supernatant, was measured using a hydrogen peroxide assay kit (Abcam). To this end, 1.0×10^5 WT, CD11b$^{-/-}$, or gp91$^{phox-/-}$ mouse primary microglia were seeded on each well of 96-well plates overnight. The cells were incubated at 37 °C for 1 h in fresh Leibovitz's L-15 media containing control vehicle, 250 nM α-syn, or a mixture of α-syn and 100 U/mL catalase. Then 50 μL of the culture supernatant was collected from each well for the H_2O_2 assay.

To measure the intracellular H_2O_2 level, 1.0×10^5 WT microglia were seeded on each well of 96-well plates in the presence or absence of 1 mg/mL catalase overnight. This allowed the delivery of catalase to the cells (11). After washing, the microglia were stimulated using Leibovitz's L-15 media containing 0.1% BSA, 250 nM α-syn, or a mixture of α-syn and 100 U/mL catalase at 37 °C for 1 h. After washing, 50 μL of media and 50 μL of AbGreen Indicator working solution (Abcam) were added to each well, and the plate was measured at 37 °C using a SpectraMax MAX GEMINIXS fluorescence reader (Molecular Devices) every 5 min for 1 h.

Statistical Analysis. All results are expressed as mean ± SEM. Statistical analyses were performed via ANOVA, followed by the Newman–Keuls multiple-comparisons test. For comparisons of two groups, the Student *t* test was used. $P < 0.05$ was considered significant.

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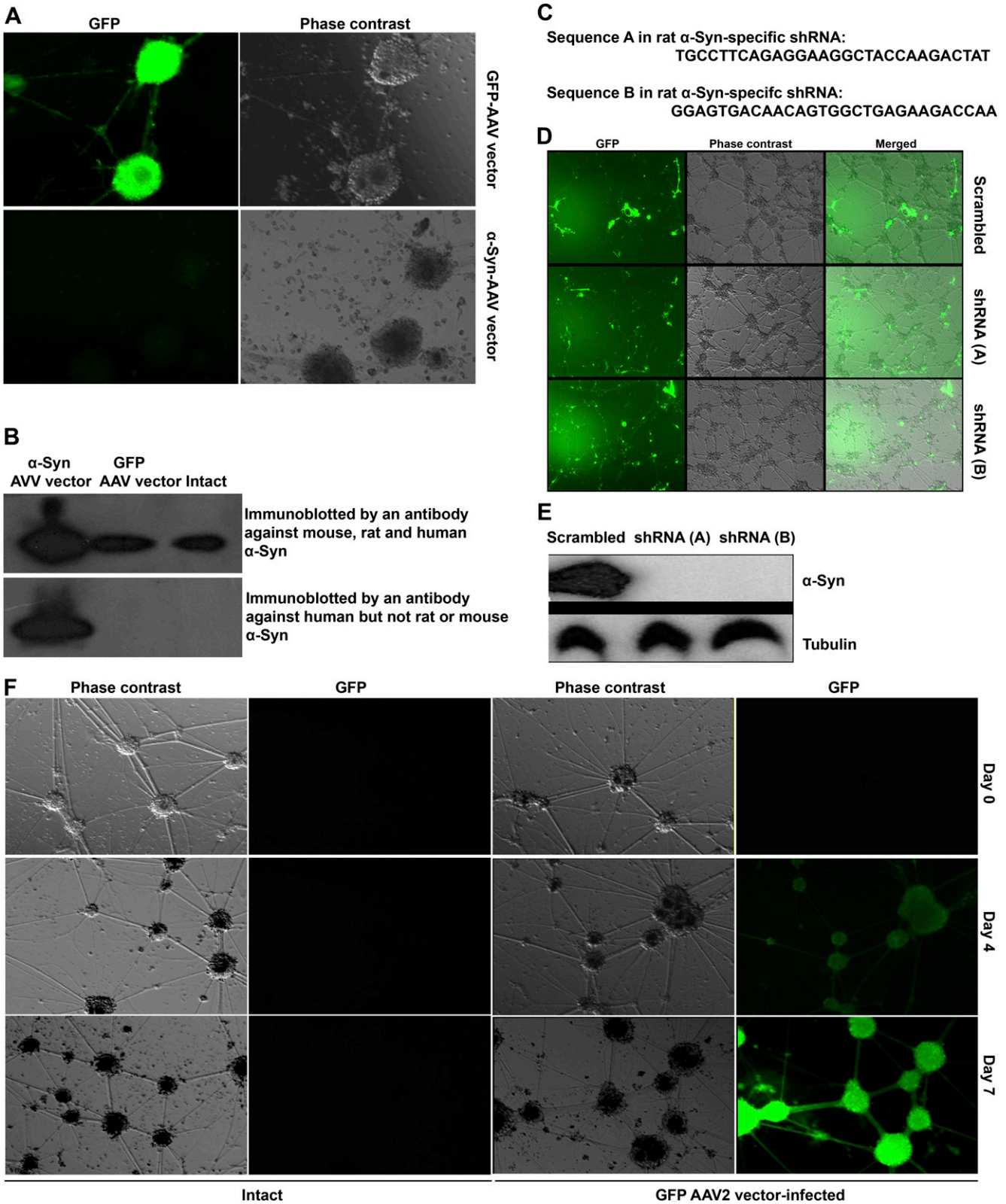

Fig. S1. Exogenous protein expression and cell morphology of rat primary neurons undergoing viral or shRNA expression manipulation. (*A*) Representative images showing the expression of exogenous proteins in rat neuron-enriched cultures infected with an AAV2 vector. The AVV2 vectors contained cDNA encoding either human α-syn or GFP, a reporter indicating that the infection efficiency was ∼100%. (*B*) Immunoblots confirming the expression of exogenous α-syn in rat neuron-enriched cultures. The level of total α-syn, which was detected using an Ab recognizing mouse, rat, and human α-syn, was markedly higher in the α-syn-AAV2–infected neuronal cultures than in the GFP-AVV2–infected control or intact cultures (*Upper*). The expression of exogenous α-syn was further confirmed using an Ab specific for human α-syn (*Lower*). (*C*) Two shRNA sequences specific to rat α-syn designed to knock down endogenous α-syn expression

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in rat primary neurons. (*D*) Representative images showing the successful infection of the rat neuron-enriched cultures using lentiviral vectors containing GFP-scrambled or rat α-syn–specific shRNA. (*E*) Immunoblots confirming the down-regulation of the expression of endogenous rat neuronal α-syn using the specific shRNA. (*F*) Representative images showing GFP expression and the changes in rat neuronal morphology over time. Typically, a fluorescence signal could be observed via microscopy on day 4 after AVV2 infection, which is equivalent to day 11 after cell seeding. By day 7 after infection (day 14 after cell seeding), stronger signals were detected, suggesting successful infection and continuous cell viability. Although the infected cells remained viable on day 14, they appeared dysmorphic, indicating an unhealthy status. In parallel, the uninfected (intact) rat neurons exhibited a similar morphology after 14 d in culture, suggesting that such an unhealthy phenotype possibly resulted from the lack of glial contacts, but was not caused by the viral infection.

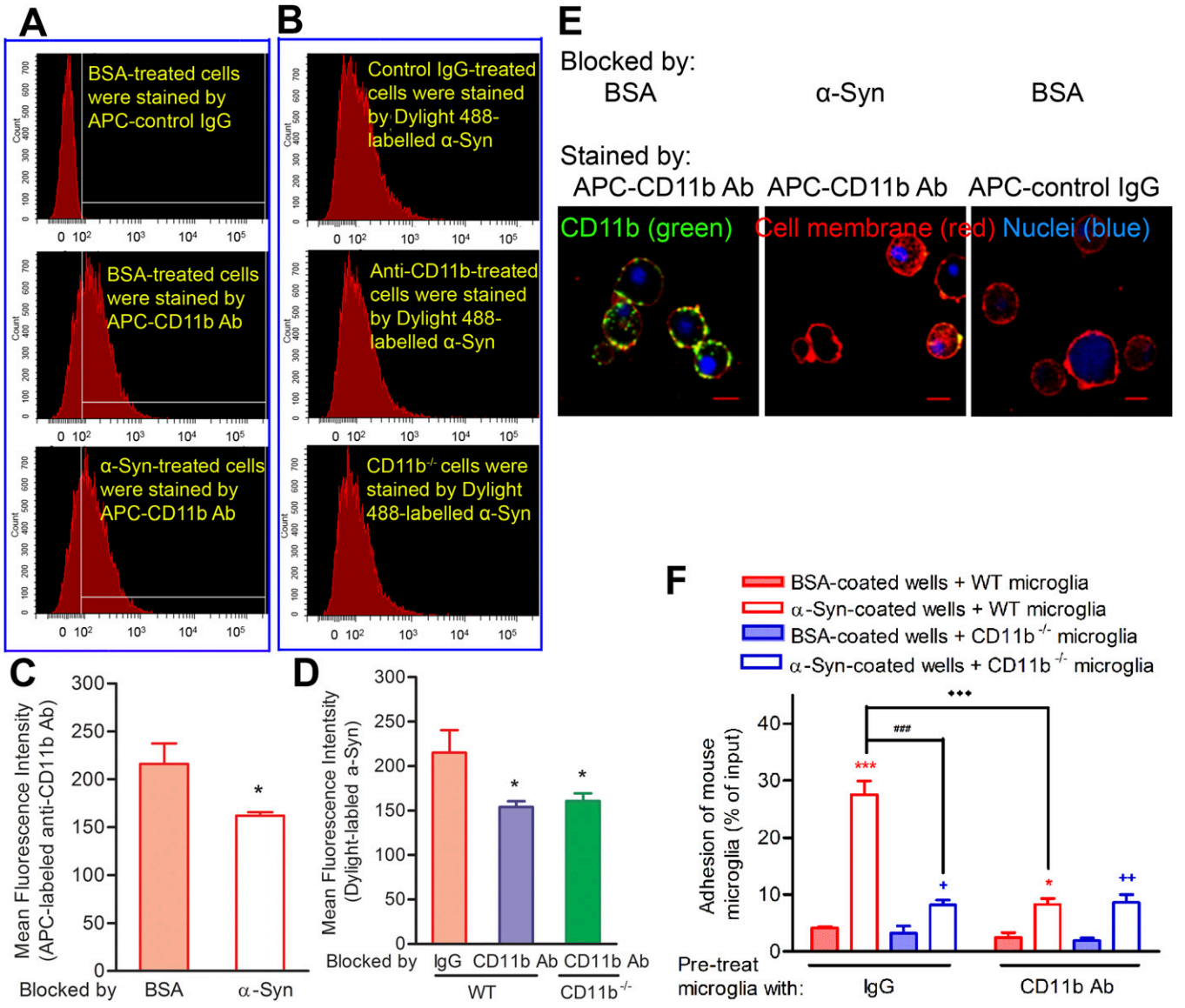

Fig. S2. Additional evidence that α-syn binds to CD11b. (*A*) Representative images showing the flow cytometric analysis of the binding of the anti-CD11b Ab to WT mouse microglia in the presence or absence of α-syn aggregates. (*B*) Representative images showing flow cytometry analysis of the binding of Dylight 488-labeled α-syn aggregates to WT or CD11b$^{-/-}$ microglia pretreated with the anti-CD11b Ab or control IgG. (*C*) Quantitative analysis of binding of the APC-labeled anti-CD11b Ab to WT mouse microglia, as represented in *A*. $n = 4$. $^{*}P < 0.05$ for comparison of BSA and α-syn treatment, Student *t* test. (*D*) Quantitative analysis of the binding of Dylight 488-labeled α-syn aggregates to WT or CD11b$^{-/-}$ mouse microglia, as represented in *B*. $n = 5$. $^{*}P < 0.05$ compared with IgG-treated WT microglia, ANOVA. (*E*) Representative confocal images showing immunostaining for CD11b on microglia. WT mouse microglia were pretreated/with BSA or α-syn and then incubated in a plasma membrane marker (Invitrogen) and an APC-conjugated anti-CD11b Ab or the control IgG. (Scale bar: 10 μm.) (*F*) Adhesion assays indicating the capacity of α-syn to bind to microglia in a CD11b-dependent manner. n =5. $^{*}P < 0.05$, $^{***}P < 0.001$, comparing WT microglial adhesion to the α-syn–coated surface with the corresponding BSA-coated control; $^{+}P < 0.05$, $^{++}P < 0.01$, comparing CD11b$^{-/-}$ microglial adhesion to the α-syn–coated surface with the corresponding BSA-coated control; $^{\#\#\#}P < 0.001$, $^{\blacklozenge\blacklozenge\blacklozenge}P < 0.001$ as indicated; ANOVA followed by the Newman–Keuls multiple-comparisons test.

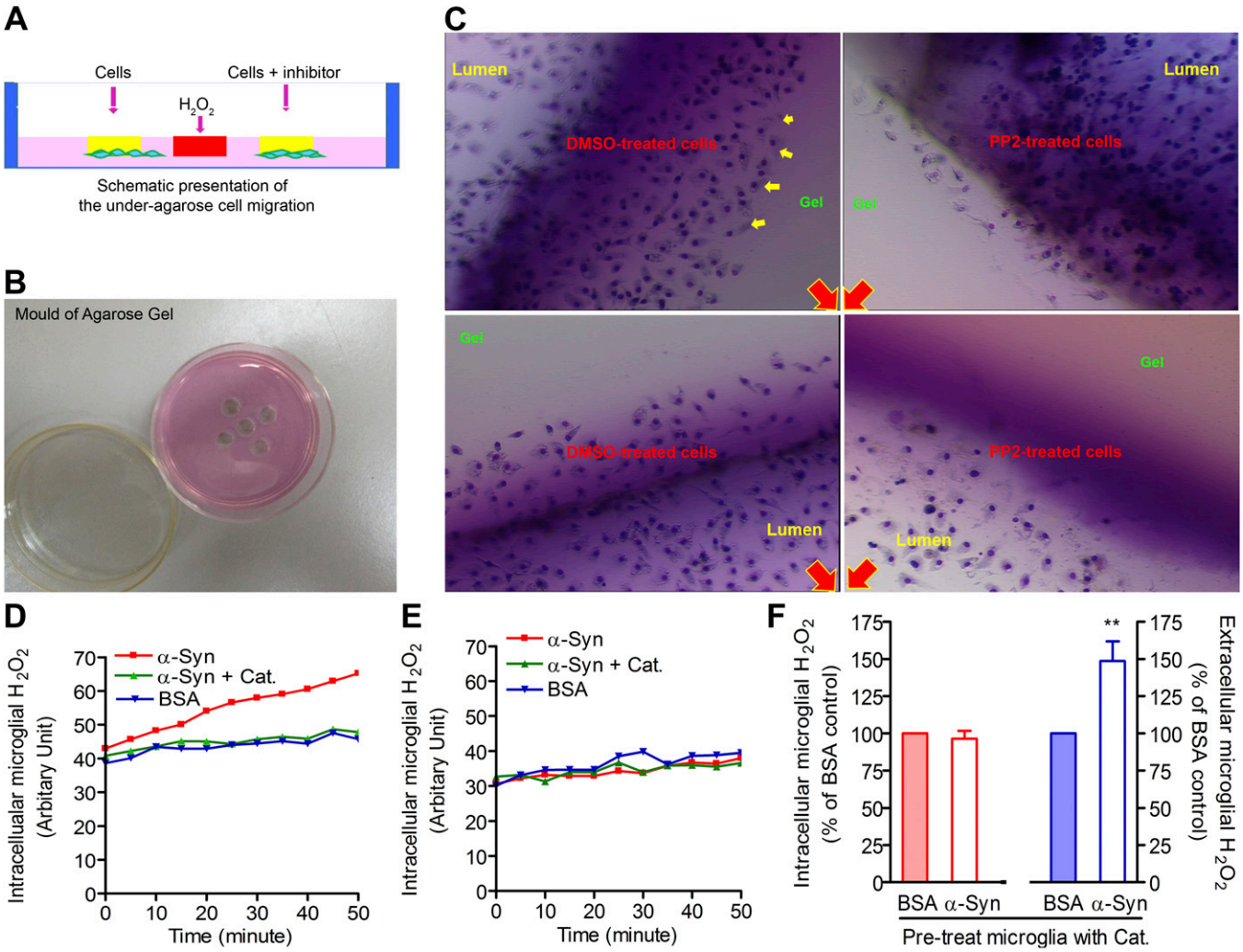

Fig. S3. Chemotaxis toward H_2O_2 was detected using an under-agarose gel migration assay and changes of intracellular and extracellular microglial H_2O_2 after stimulation of α-syn. (*A*) Schematic diagram of the under-agarose gel migration assay. The chemoattractant H_2O_2 was added to the central well, and mouse or rat primary microglia or rat microglia-derived HAPI cells were loaded in the peripheral wells containing DMSO or the Src inhibitor PP2. (*B*) Photograph showing the mold used to produce the agarose gels. (*C*) Images showing an example of an under-agarose gel migration assay and quantitative analysis. The term "lumen" indicates the position inside a well, whereas "gel" refers to the body of the gel. The yellow arrows indicate the leading edge of the cells migrating in the gels. The red arrows indicate the source of the chemoattractant H_2O_2. The image on the top left shows that the cells on the same side of the chemoattractant (i.e., H_2O_2) migrated toward H_2O_2. In contrast, the image on the bottom left shows that the cells on the opposite side of the chemoattractant (i.e., H_2O_2) migrated away from H_2O_2. The corresponding images on the right represent cell migration in the presence of PP2. The net value of chemotaxis was calculated as the difference between the migrating cells on the same side and the opposite side of the chemoattractant. (*D*) Representative curve showing dynamic changes in the intracellular H_2O_2 concentration in WT mouse microglia, which were stimulated by BSA or α-syn with or without catalase (Cat). (*E*) Representative curve showing dynamic changes in the intracellular H_2O_2 concentration in catalase-pretreated microglia, which were stimulated by BSA or α-syn with or without catalase. (*F*) Changes in the concentration of extracellular and intracellular H_2O_2 after pretreatment with catalase and stimulation by BSA or α-syn with or without catalase. The extracellular H_2O_2 in culture supernatant and the intracellular H_2O_2 were measured using an Abcam kit and a specific indicator. $n = 4$. The Student *t* test was performed.

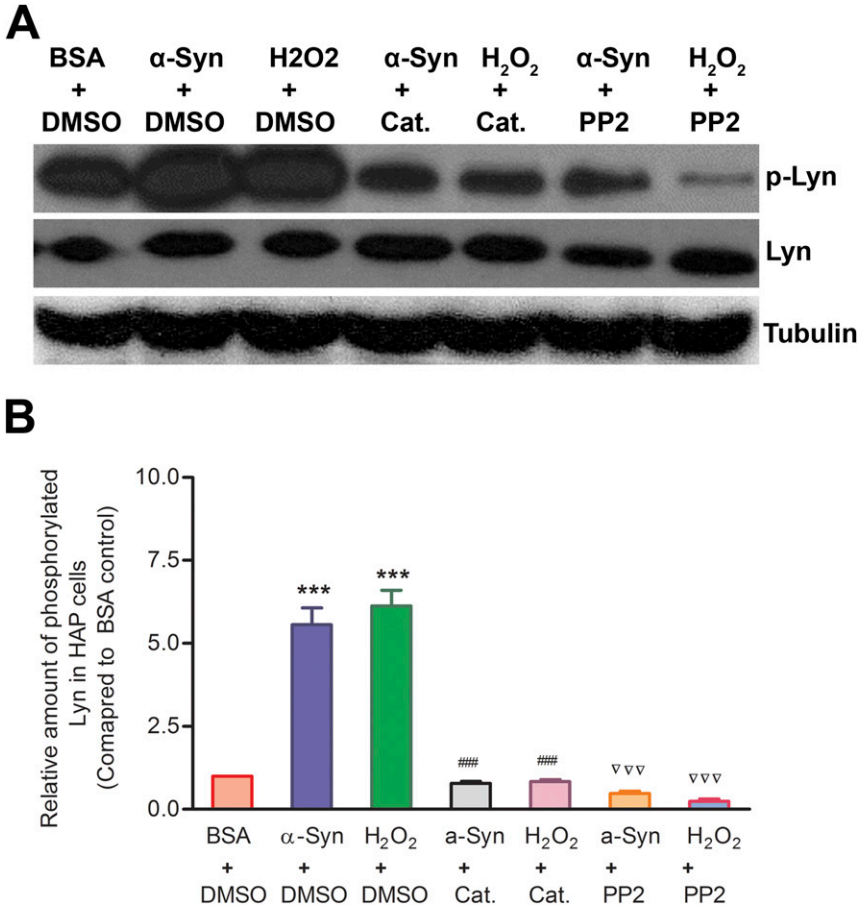

Fig. S4. Either α-syn or H_2O_2 stimulates HAPI cells to phosphorylate Lyn, which is attenuated by either catalase or the inhibitor PP2. (*A*) Representative immunoblots showing the changes in Lyn phosphorylation in HAPI cells that were stimulated with either α-syn or H_2O_2 in the presence or absence of catalase (Cat) or PP2. (*B*) Statistical summary of three independent experiments performed as described in *A* were performed. $^{***}P < 0.001$ compared with the BSA-treated control; $^{\#\#\#}P < 0.001$ and $^{\triangledown\triangledown\triangledown}P < 0.001$ compared with each corresponding group in which the microglia were stimulated with DMSO and either α-syn or H_2O_2; ANOVA followed by the Newman–Keuls multiple-comparisons test.

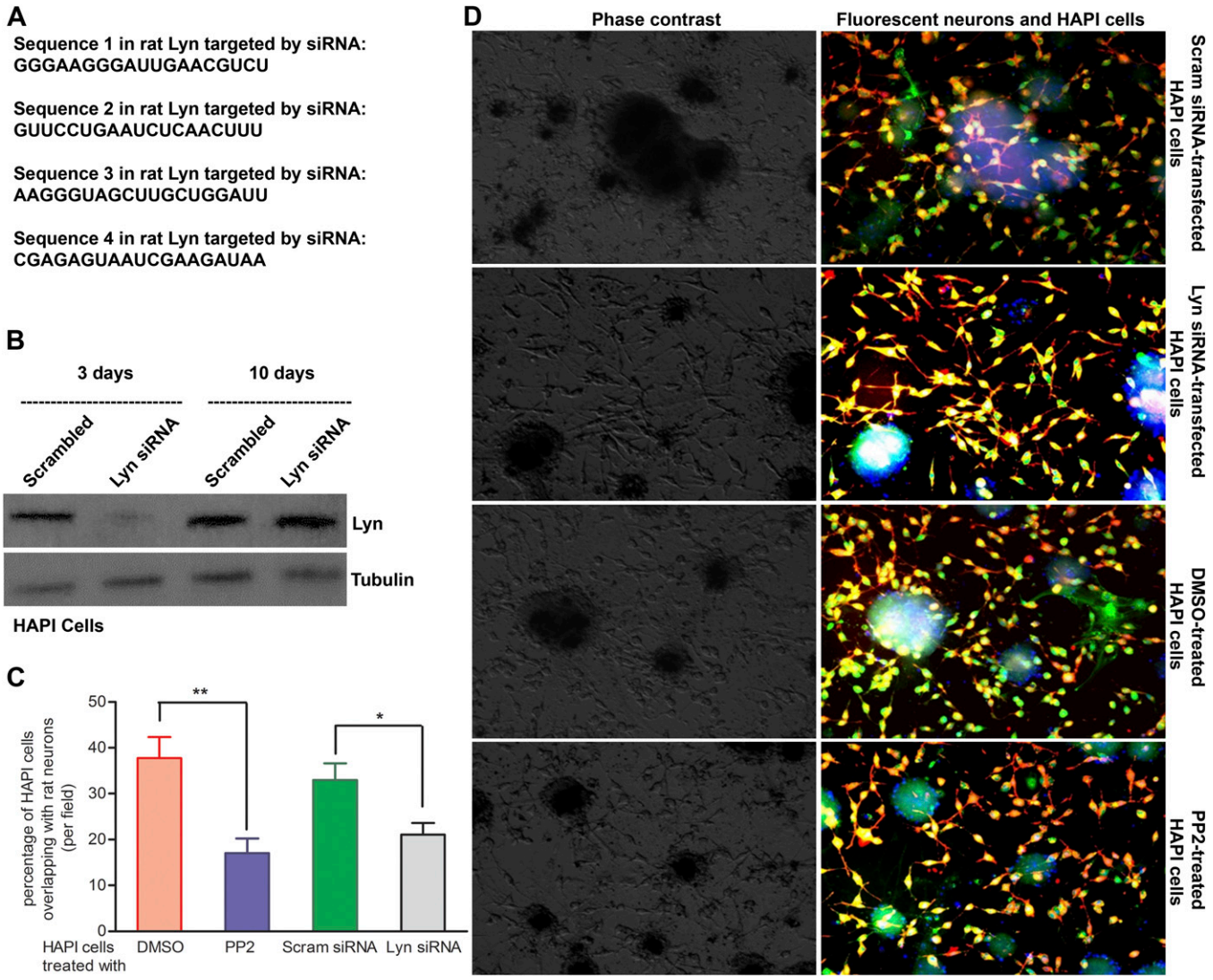

Fig. S5. Down-regulation of Lyn expression in HAPI cells by siRNA and relative location of Lyn siRNA- or PP2-treated HAPI cells and rat primary neurons after overnight coculture. (*A*) Four sequences of rat Lyn that were targeted using specific siRNA. (*B*) Expression of Lyn in HAPI cells on day 3 and day 10 after transfection with siRNAs. (*C*) Quantitative analysis of the colocalization of HAPI cells with rat primary neurons after overnight coculture. $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$ as indicated, Student *t* test. (*D*) Representative images showing relative locations of the rat primary neurons and HAPI cells after overnight incubation in the presence or absence of scrambled (scram) or Lyn siRNA or the Src inhibitor PP2.

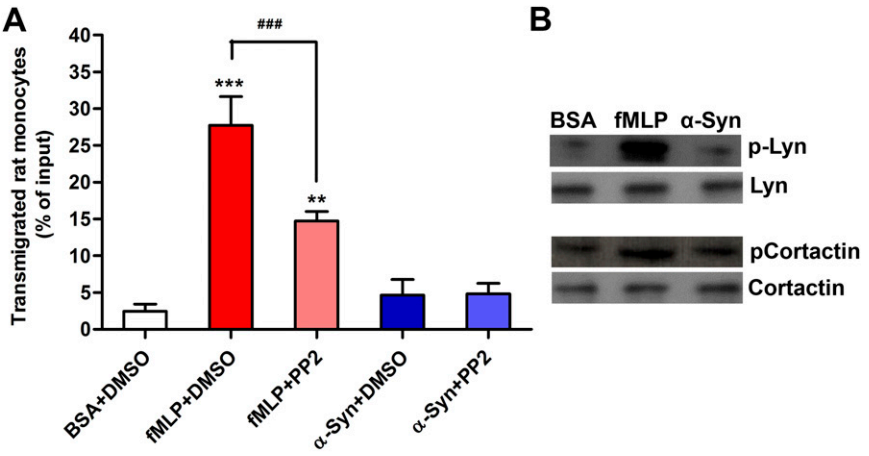

Fig. S6. Responses of rat primary monocytes to the challenge with fMLP or α-syn. (*A*) Effect of PP2 on migration of rat primary peripheral blood monocytes toward purified α-syn aggregates or fMLP. $n = 4$. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the BSA control; $^{\#\#\#}P < 0.001$ compared as indicated; ANOVA followed by the Newman–Keuls multiple-comparisons test. (*B*) Phosphorylation of Lyn and cortactin in rat primary peripheral blood monocytes on the stimulation of BSA, fMLP, or α-syn.

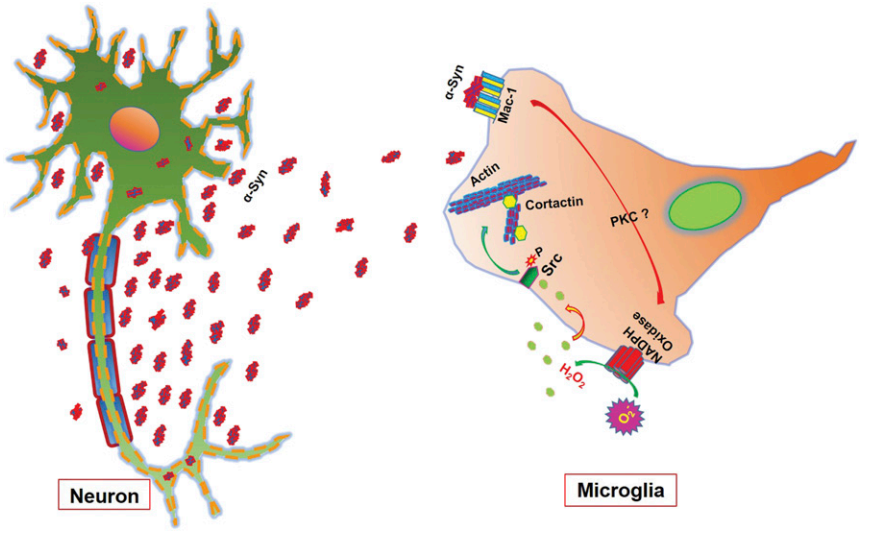

Fig. S7. Schematic of the mechanism by which neuron-derived α-syn induces the directional migration of microglia toward neurons. Even under physiological conditions, neurons release some α-syn into the interstitial tissue; however, this release is amplified when neurons are injured or become unhealthy or when mutated α-syn accumulates intracellularly. The released α-syn, predominantly in an aggregated form (e.g., oligomers), establishes a gradient in the space between the neurons and the microglia. α-syn aggregates may bind directly to the CD11b (i.e., Mac-1) on microglia, eliciting an undetermined inside-out signal to activate Nox2. Activation of Nox2 results in the generation of O_2^-, which is promptly transformed into H_2O_2. This reaction leads to a relatively high concentration of H_2O_2 in the microenvironment surrounding the leading edge of the microglia. This H_2O_2 readily permeates plasma membranes and diffuses into the cytosol, leading to changes in the intracellular H_2O_2 concentration. The Src family kinase Lyn senses the changes in the intracellular H_2O_2 levels and becomes phosphorylated. Lyn activation induces the phosphorylation of the actin-associated protein cortactin, causing actin skeleton rearrangement. Collectively α-syn acts as a chemoattractant to recruit microglia in a manner that is dependent on the generation and intracellular diffusion of H_2O_2. Lyn acts as a sensor to transduce the intracellular signals initiated by H_2O_2 to phosphorylate cortactin, inducing rearrangement of the actin skeleton in microglia and driving microglial migration. The α-syn gradient in the space between the neurons and the microglia continuously stimulates the aforementioned cascade of reactions in the microenvironment surrounding the leading edge of the microglia as well as in the cytosol. As a result, the microglia continue to migrate along the concentration gradient of α-syn toward the sources of α-syn.