Supplementary Information

Organic nanoparticles with aggregation-induced emission for tracking bone marrow stromal cell in the rat ischemic stroke model

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Experimental Section

Materials. 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}) was a gift from Lipoid GmbH (Ludwigshafen, Germany). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000] (DSPE-PEG_{2000}-NH_2) was a commercial product of Avanti Polar Lipids, Inc. Qtracker® 655 cell labeling kit was purchased from Life Technologies, Invitrogen, Singapore. Fluoromount® aqueous mounting medium, tetrahydrofuran (THF), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), endothelin-1 (ET-1), penicillin-streptomycin solution, and trypsin-EDTA solution were purchased from Sigma-Aldrich. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were bought from Thermo Scientific, Singapore. Tween-20 was purchased from Sinopharm Chemical Reagent Co, Ltd. Cell penetrating peptide derived from transactivator of transcription proteins, HIV-1 Tat (49-57) with C-terminus modified with cysteine (RKKRRQRRRC), was a commercial product customized by GenicBio, China. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Breford, USA).

Characterization. The UV-Vis spectra were recorded on a Milton Roy 5 Spectronic 3000 Array spectrophotometer and the fluorescence spectra were measured using a fluorometer (LS-55, Perkin Elmer, USA). The average particle size and size distribution were determined by laser light scattering with a particle size analyzer (90 Plus, Brookhaven Instruments Co. USA) at a fixed angle of 90° at 24 °C. The morphology of AIE NPs was studied by high-resolution transmission electron microscope (HR-TEM, JEM-2010F, JEOL, Japan).

BMSC isolation. The rat bone marrow stromal cells (BMSCs) were isolated according to the following protocol. All animal experiments were performed in compliance with guidelines set by

the the Institutional Animal Care and Use Committee (IACUC), National University of Singapore.

The rat was sacrificed with high dose of pentobarbital, and femurs and tibias were aseptically dissected. Both ends were cut to extrude the bone marrow with cold DMEM High-Glucose (HyClone). This was achieved by flushing the bone marrow with a 21-guage needle until the bone appeared white. Red clumps of cells were dispersed by repeated flushing with the needle and then passed through a 100 μm Nylon cell strainer into a 50 mL falcon tube. The collected cell suspension was spun in a centrifuge for 5 minutes, 300g at 18 °C. Subsequently, the pellet was collected and suspended in growth medium DMEM High-Glucose supplemented with 10% FBS (Gibco) and 1% penicillin-streptomycin and reseeded onto 75 cm^2 tissue culture flasks. The medium was replaced on Day 1 and 4 from isolation (Day 0), with medium change every two days afterwards. Attaching adherent cells from passage 3 and 5 (P3 and P5) were then screened for various mesenchymal stem cell markers.

Immunofluorescence staining of BMSC surface biomarkers. Rat BMSCs were fixed with 4% paraformaldehyde for 15 minutes. After washing, cells were blocked with 2% skim milk for 1 h at room temperature. Samples were then incubated with the respective primary monoclonal antibodies (mAb) overnight at 4 °C, including biotinylated hamster anti-CD29 mAb, biotinylated mouse anti-CD45 mAb, and mouse anti-CD 90 mAb, respectively. The cells were then incubated with fluorescent conjugate-secondary antibodies for 1 h at room temperature. Alexa Fluor® 488 Streptavidin is used for biotinylated CD29 and CD45 mAb, Alexa Fluor® 488 conjugated goat anti-mouse antibody is used for anti-CD90 mAb. Samples with omitted primary antibodies were used as negative controls. After counterstaining with DAPI, the slides were mounted with Prolong Gold Antifade Reagent, left to dry and later photographed with a fluorescence

microscope. Besides fluorescence imaging, flow cytometry tests were also performed to evaluate the surface marker expressions of P5 BMSCs. Live cells were suspended in 0.5% BSA and 2mM EDTA maintained at close to 4 °C. Cells were incubated with the primary antibodies at room temperature: biotin hamster anti-CD29, mouse anti-CD90 and biotinylated mouse anti- CD45 for 20 minutes. After washing, cells were incubated with the respective secondary antibodies: either with pairing of Alexa Fluor® 488 Streptavidin conjugates and Alexa Fluor® 546 goat anti-mouse or Alexa Fluor® 647 Streptavidin conjugates and Alexa Fluor® 488 goat anti-mouse for 20 minutes. Isotype controls for each secondary antibody were prepared accordingly with the primary antibodies omitted. A negative control was prepared for each run with unstained cells. After washing twice, flow cytometric analysis was performed using the CyAn-ADP analyzer (Beckman Coulter, Inc). Live events (10, 000 cells per loaded sample) were acquired for analysis with Summit V4.3.02 Build 2451 software (Beckman Coulter, Inc).

In vitro cell tracking. Rat BMSCs (P5) were cultured in 6-well plates (Costar, IL, USA) to achieve 80% confluence. After medium removal and washing with PBS, 2 nM Tat-AIE NPs or Qtracker® 655 in DMEM medium were then added to the wells. After 12 h incubation at 37 °C, the cells were washed twice and detached by tripsin and resuspended in culture medium. Upon dilution, the cells were subcultured in 6-well plates for designated days, respectively. After designated time intervals, the cells were washed twice and then trypsinalized to suspend in PBS buffer. The fluorescence intensities of cells were then analyzed by flow cytometry measurements using Cyan-LX (DakoCytomation) and the histogram of each sample was obtained by counting 10,000 events (λ_{ex} = 488 nm, 680/30 nm bandpass filter). For confocal image studies, the cells for flow cytometry analysis were used for fluorescence images obtained by a Leica TCS SP 5X.

The laser at 514 nm (1 mW) was adopted to obtain the one-photon excited fluorescence images with a 550–800 nm bandpass filter.

Cytotoxicity of Tat-AIE NPs. The metabolic activities of rat BMSCs (P5) were evaluated using methylthiazolyldiphenyltetrazolium bromide (MTT) assays. Cells were seeded in 96-well plates (Costar, IL, USA) at a density of 6×10^4 cells/mL. After 24 h incubation, the old medium was replaced by Tat-AIE NP suspension at concentrations of 2, 4 and 6 nM, and the cells were then incubated for 72 h. To eliminate the UV-Vis absorption interference of the Tat-AIE NPs at 570 nm, the cells incubated with the Tat-AIE NPs without post-treatment by MTT were used as the control. After 72 h, the wells were washed twice with PBS buffer and 100 μL of freshly prepared MTT (0.5 mg/mL) solution in culture medium was added into each well. The MTT medium solution was carefully removed after 3 h incubation in the incubator. DMSO (100 μL) was then added into each well and the plate was gently shaken for 10 min at 24°C to dissolve all the precipitates formed. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan) and cell viability was expressed by the ratio of the absorbance of the cells incubated with Tat-AIE NP suspension to that of the cells incubated with culture medium only.

Rat stroke model. Male Wistar rats were anesthetized with ketamine (75 $mg \cdot kg^{-1}$, intraperitoneally; Parnell Laboratories Pty Ltd, Alexandria, NSW, Australia) and xylazine (10 $mg \cdot kg^{-1}$, i.p.; Troy Laboratories Pty Ltd, Smithfield, NSW, Australia). The stroke model was created by topical application of 25 μg/μL of ET-1 to the rat brain (0.5 mm posterior and 2.5 mm anterior to bregma). To ensure uniformity, a stereotaxic frame was used to mount the rat. A syringe containing the ET-1 to be delivered was mounted on the same frame. During the operation, 5 μL of ET-1 in total was placed onto the specified location, starting with 0.5 μL of

ET-1 to each of five points per 1 minute interval, i.e. each point has 1 μL topical lesion, leaving 10 minutes for the last application.

Transplantation of Tat-AIE NP-labeled BMSCs. Rat BMSCs were cultured in a 75 cm^2 tissue culture flasks to achieve 80% confluence. After medium removal and washing with PBS, 2 nM Tat-AIE NPs in DMEM medium were then added to the flask. After 12 h incubation at 37 °C, the cells were detached by tripsin and resuspended in DMEM to achieve a final concentration of 2×10^6 cells/mL. At 1 day post-ischemia, animals were anesthetized and the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed. ECA,the branch of ICA and the pterygopalatine artery, were ligated close to its origin. The Tat-AIE NP-labeled BMSCs were then transferred to a polyethylene catheter (PE-10). The catheter was advanced through the small puncture from CCA into the luman of ICA. Approximately of 1 million BMSCs labelled with Tat-AIE NPs were infused at a flow rate of 3 mL/h (B Braun Perfusor® compact). All the transplantation procedures were performed under aseptic conditions.

Histology of the brain tissue. One week after transplantation of BMSCs labeled with Tat-AIE NPs, the rats were perfused with PBS. Brains were extracted for histology staining. Forty μm thickness sections were prepared with a cryostat and stained by thionin to verify the lesion site. For the confocal imaging, adjacent sections were stained with DAPI and imaged by confocal microscopy (Leica TCS SP 5X). Upon excitation at 405 nm, the fluorescence of Tat-AIE NPs and DAPI were collected through 600–800 nm and 420–500 nm bandpass filters, respectively.

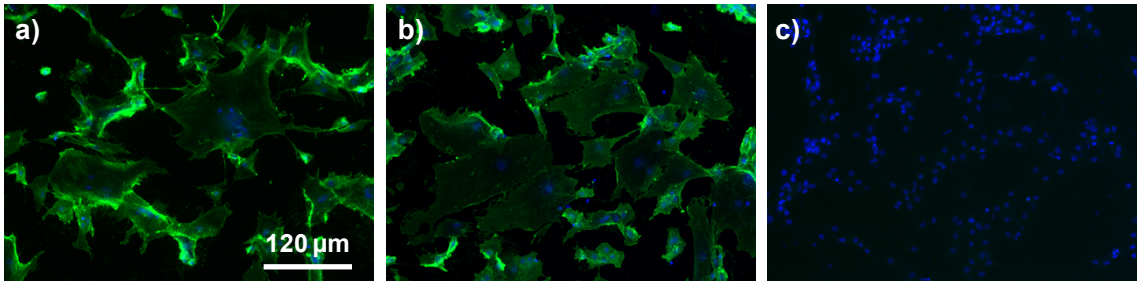

Figure S1. Immunofluorescence imaging of P5 BMSCs for surface markers: (a) CD29, (b) CD90, and (c) CD45, respectively. Cell nuclei were stained by DAPI. All images share the same scale bar.

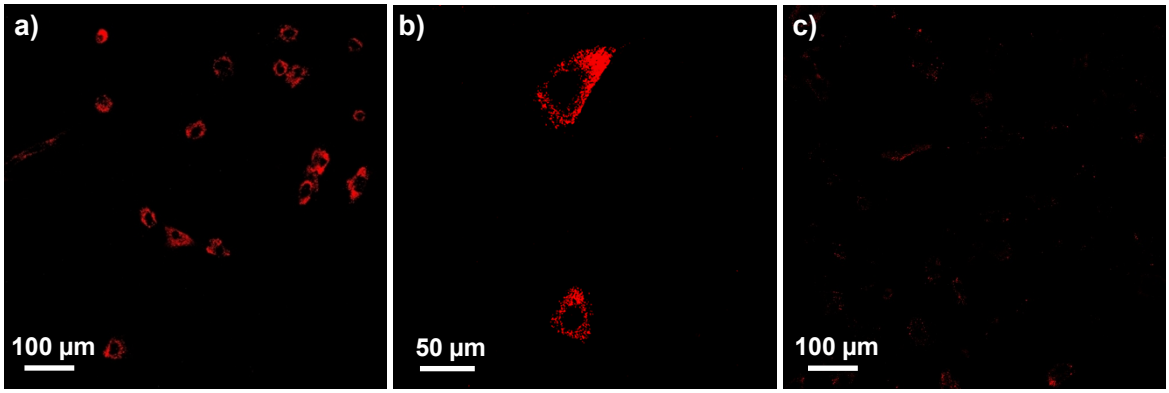

Figure S2. Confocal images of BMSCs after incubation with 2 nM (a, b) Tat-AIE NPs and (c) AIE NPs without functionalization of Tat peptide for 4 h at 37 °C (λ_{ex} = 514 nm, 550–800 nm bandpass filter).

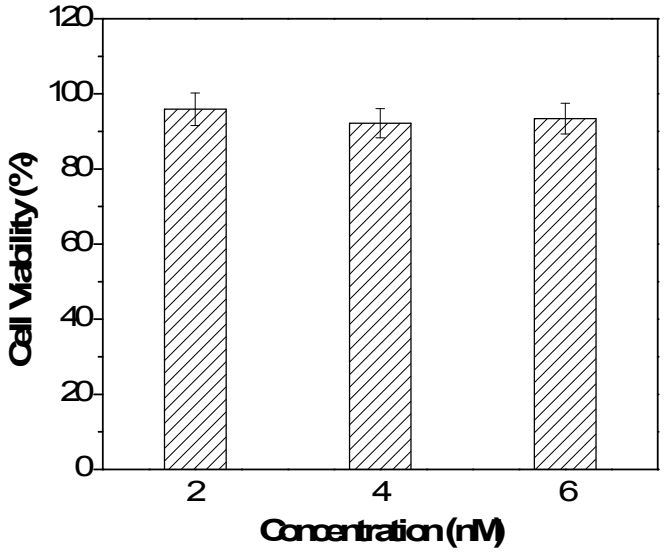

Figure S3. Metabolic viability of BMSCs after incubation with 2, 4, and 6 nM Tat-AIE NPs for 48 h.

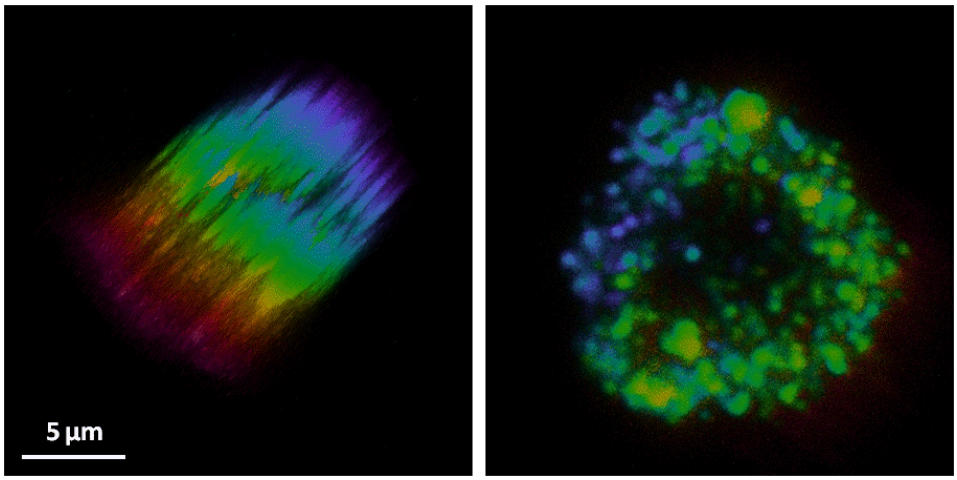

Figure S4. Color-coded projections of z-stacks of fluorescence images of the suspended BMSCs labeled with Tat-AIE NPs. The images were taken under excitation at 514 nm (~1 mW) with a 550–800 nm bandpass filter. All images share the same scale bar.