Supported Information

Magnetic ternary nanohybrids for nonviral gene delivery of stem cells and applications on cancer therapy

Rih-Yang Huang, Yee-Hsien Lin, Ssu-Yu Lin, Yi-Nan Li, Chi-Shiun Chiang, and Chien-Wen Chang

Material and methods

FITC-HA-SPIO Synthesis

FITC modified HA (FITC-HA) was first synthesized for preparing FITC-HA-SPIO. 100 mg HA were dissolved in 4 mL formamide with gentle agitation for 12 hours. 50 mg of sodium bicarbonate (Sigma-Aldrich), 50 mg of dibutyltin dilaurate (TCI) and 100 mg FITC (Sigma-Aldrich) were dissolved in 2.5 mL DMSO and added slowly into HA solution. The mixture was stirred on a steam bath for 30 minutes. The reactant was then dialysis against water for 2 days and subjected to lyophilization. The product was reconstituted in deionized water and precipitated in ethanol to remove remaining FITC. Ligand exchange process, mentioned previously, was used to modified FITC-HA into OA-SPIO. To ensure the successful preparation of FITC-HA-SPIO, same amount of HA-SPIO or FITC-HA-SPIO were suspended in deionized water and the fluorescence spectrum of each sample was characterized.

Cellular uptake

To monitored the internalized SPIO, FITC-HA-SPIO was used to prepare MTN. Fifty thousand of hMSCs were seeded on the glass coverslips in 24-well plates and cultured at 37℃; 5% CO_2 overnight. ^{PAE}MTN using FITC-HA-SPIO was prepared and transfecting hMSCs as previously described. Afterward, the cells were washed with PBS twice. 4 or 24 hours after transfection, the cells were stained with LysoTracker™ Red DND-99 (ThermoFisher) at 300 nM for 30 minutes. Cells were then washed with PBS twice and fixed by 4% PFA/PBS for 10 minutes. The cell nucleus was stained by Hoechst 33342. The cover slip was mounted on the microslide and sealed with nail polish. The fluorescence cell imaging was visualized by CLSM (LSM 510, Zeiss)

Biodistribution

The animals were handled in accordance to the animal use protocol of the National Tsing Hua University, Hsinchu, Taiwan. The mice were orthotopic inoculated human glioma cells (U87MG cells) as described

previously. $2x10^5$ Luciferase expressed hMSCs (^{Luc}hMSCs) in 2 μL serum free F-12 medium were intracranial injected or ^{Luc}hMSCs in 100 μL PBS were intravenous injected into each nude mouse. At 4, 24, 48 and 168 hours post-injection. The mice were intraperitoneal injected with *D*-luciferin (Promega) at injection dose of 125 mg/kg. The in vivo bioluminescent imaging was recorded at 10 minutes after *D*-luciferin injected.

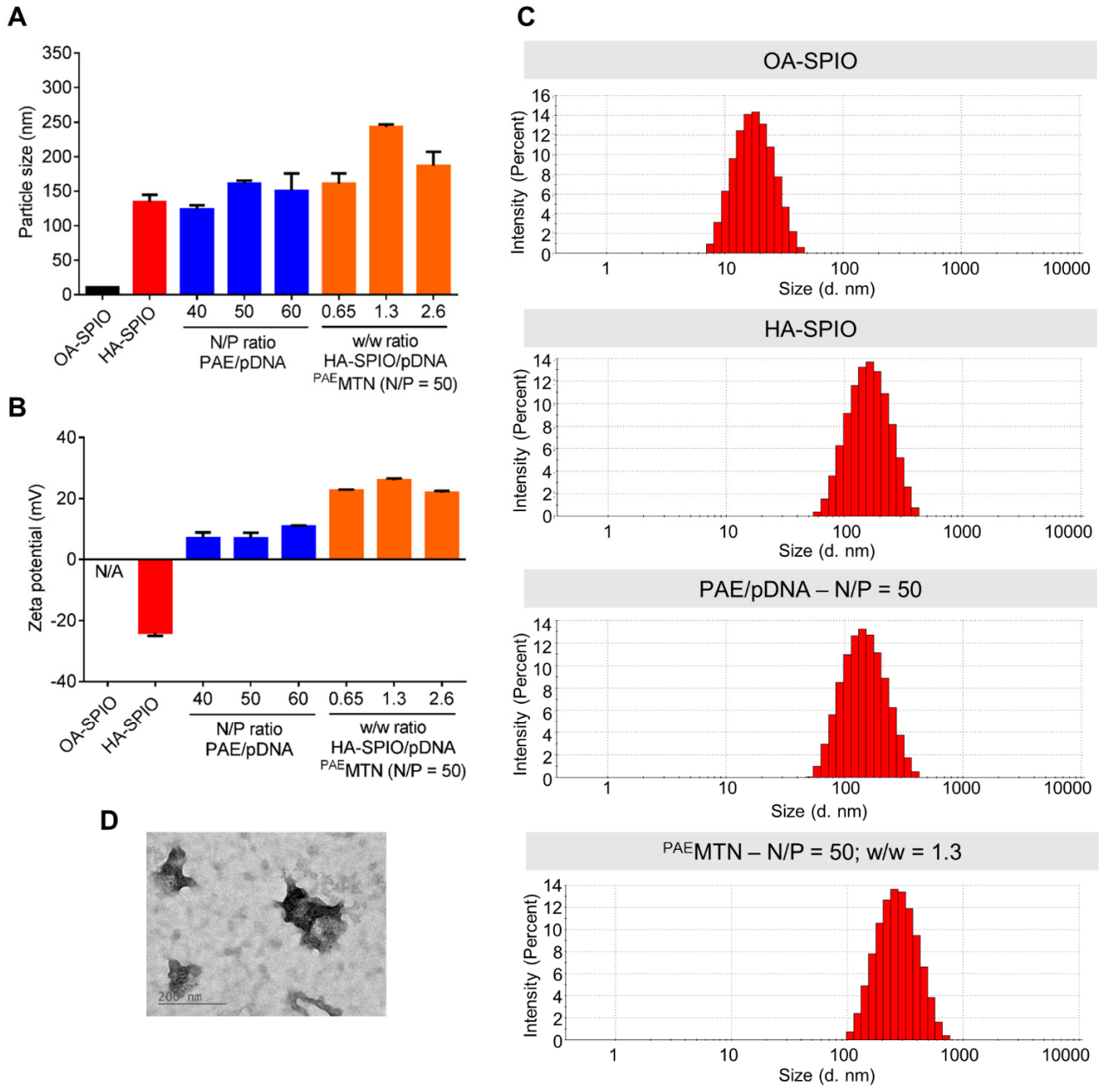

Figure S1. Physicochemical characterizations of ^{PAE}MTN. (A) Particle size and (B) Zeta potential of OA-SPIO, HA-SPIO, PAE/pDNA polyplex and ^{PAE}MTN in aqueous phase at various N/P ratios. (C) The size histogram of OA-SPIO, HA-SPIO, PAE/pDNA polyplex and ^{PAE}MTN. (D) TEM imaging of ^{PAE}MTN at N/P ratio of 50 complexed with HA-SPIO at weight ratio (HA-SPIO/pDNA) of 1.3. Data are mean±s.e.m. Data were measured from three independent experiments.

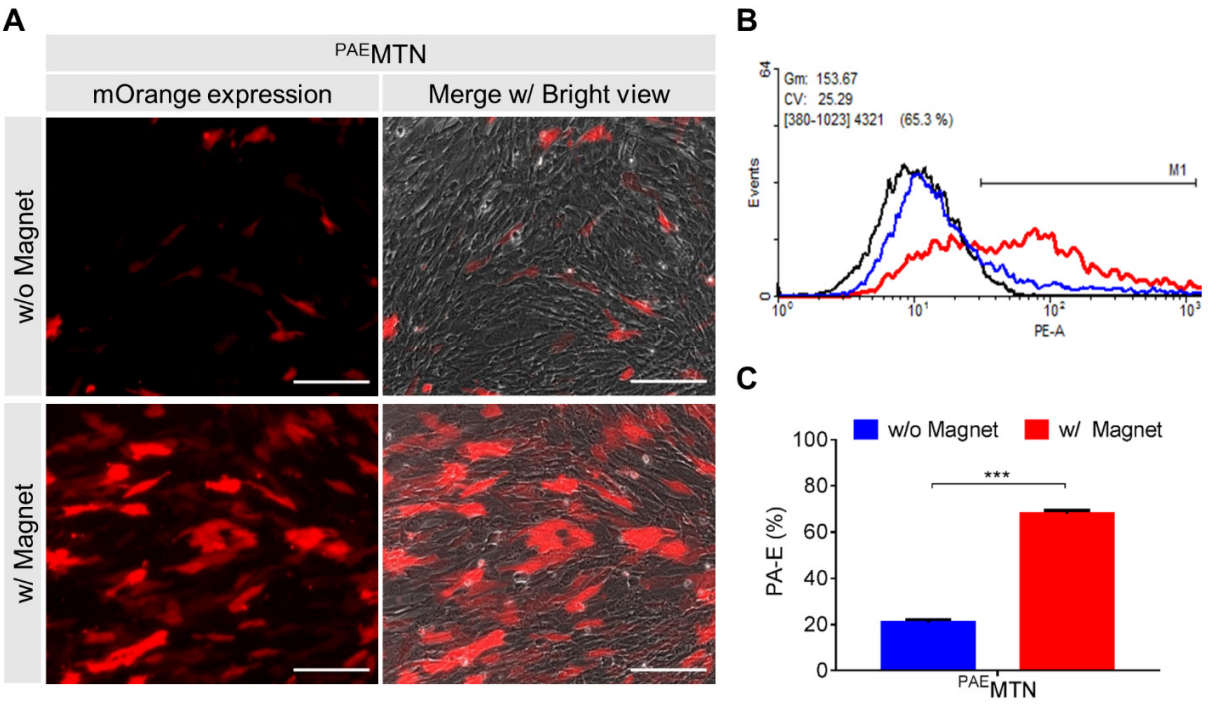

Figure S2. Transfection efficiency of ^{PAE}MTN on hMSCs by FACS analysis. The pcDNA3.mOrange expression vector was used as reporter gene for evaluating the transfection efficiency. (A) fluorescence images of hMSCs after transfection for 48 hours. (B) The histogram of hMSCs transfected by ^{PAE}MTN. The colors of lines are represented as black: no-treatment; blue: MTN without magnetic field; red: MTN with magnetic field. (C) Quantitation of transfection efficiency of MTN on hMSCs. Data are mean±s.e.m. Data were measured from three independent experiments. *P<0.05, **P<0.01, and ***P<0.001 by student's t-test.

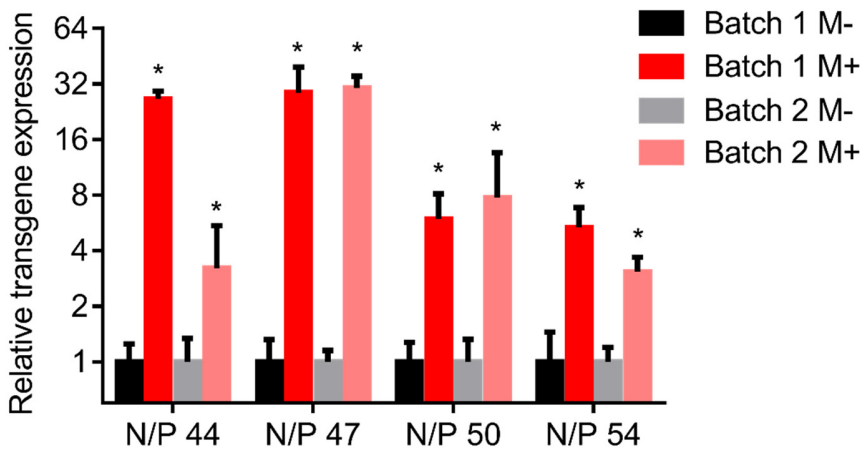

Figure S3. Comparison of transfection efficiency of ^{PAE}MTN on hMSCs from different donors (batch 1 & 2). The transgene level was quantitated and normalized by luciferase assay. M-: without magnetic attraction. M+: with magnetic attraction. Data are mean±s.e.m. Data were measured from three independent experiments. *P<0.05 by student's t-test.

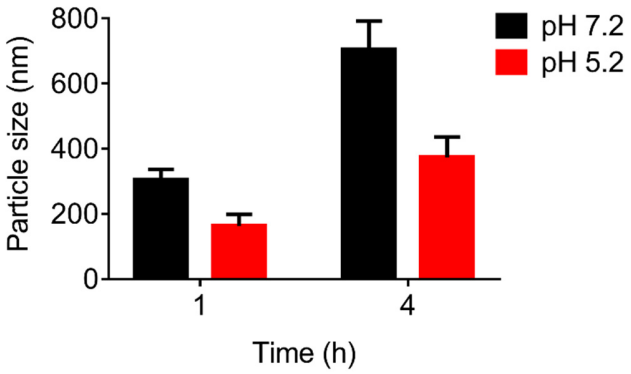

Figure S4. Colloidal stability of PAE/DNA polyplex in neutral or acidic condition. The fresh prepared PAE/DNA (N/P at 60) were incubated at neutral pH water or acetate buffer (pH 5.2) at 37℃ for 1 hour or 4 hours before particle size measurement by DLS. Data are mean±s.e.m. Data were measured from three independent experiments.

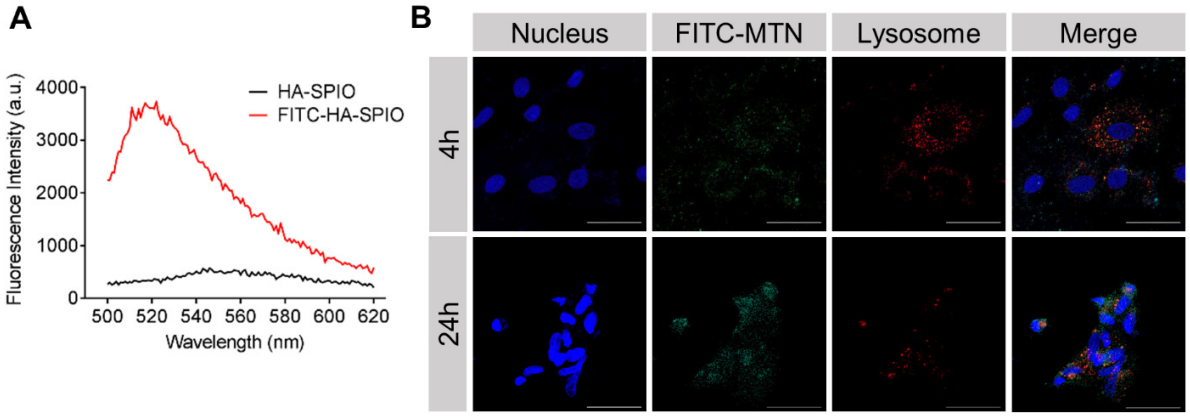

Figure S5. Intracellular trafficking study of FITC-MTN. (A) The fluorescence spectrum of HA-SPIO and FITC-HA-SPIO at excitation wavelength of 480 nm. (B) Cellular uptake of ^{PAE}MTN using FITC-HA-SPIO were visualized by confocal laser scanning microscopy (LSM 510, Zeiss). The green and red fluorescence represented the intracellular location of HA-SPIO and endosome, respectively. Nucleus were stained with Hoechst 33342 Scale bar in (B): 50 μm.

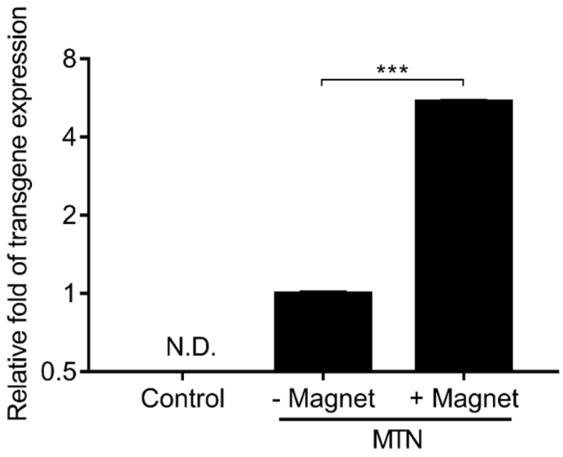

Figure S6. Real time PCR analysis of TRAIL mRNA expression from ^{MTN}hMSCs with or without applying external magnetic field. Forty-eight hours after transfection, the cells were collected, lyse for mRNA extraction and analysis. post-transfection. Data are mean±s.e.m. Data were measured from three independent experiments. *P<0.05 by student's t-test.

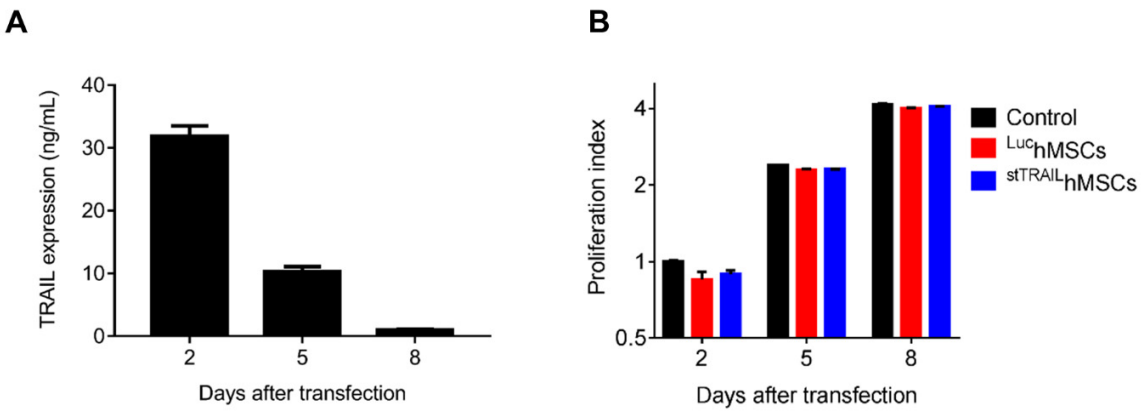

Figure S7. Time course TRAIL expression and cell proliferation of MTN-transfected hMSCs. (A) TRAIL was quantitatively analyzed by ELISA. (B) Proliferation rate of the $^{MTN/TRAIL}$hMSCs was evaluated by DNA assay. Data are mean±s.e.m. Data were measured from three independent experiments.

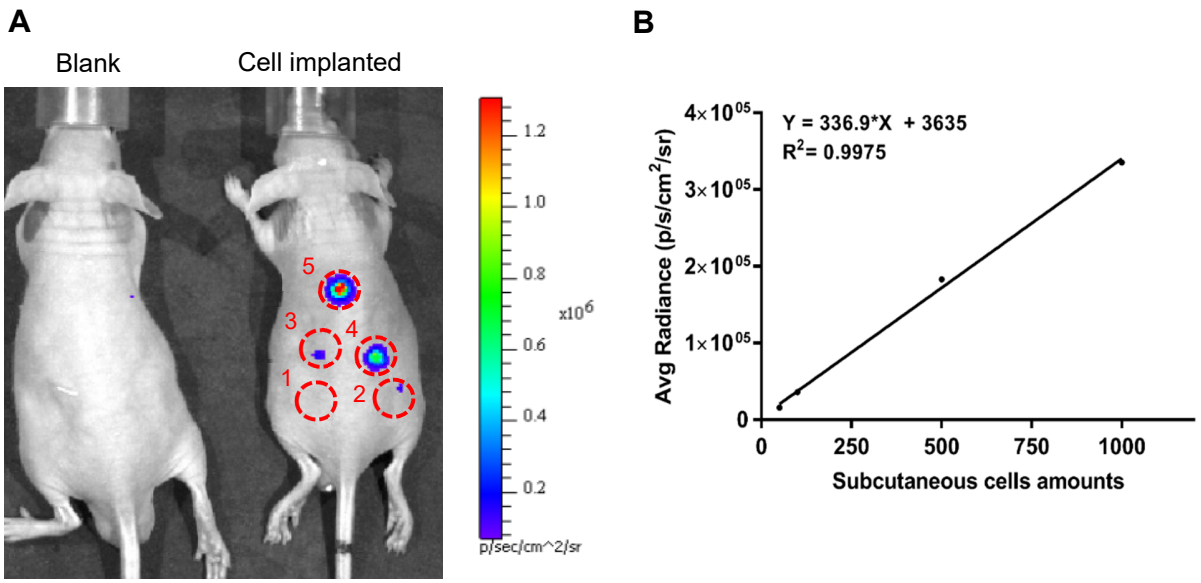

Figure S8. IVIS detection of various number of luciferase-stem cells (^{luc}hMSCs) in vivo. (A) The nude mouse was subcutaneously injected with ^{luc}hMSCs at defined cell amounts of 10 cells (No.1), 50 cells (No.2), 100 cells (No.3), 500 cells (No.4) and 1000 cells (No.5). The bioluminescent images were recorded at 4 hours after cells injection. (B) The graph of cells amounts to signals showed great proportional correlation.

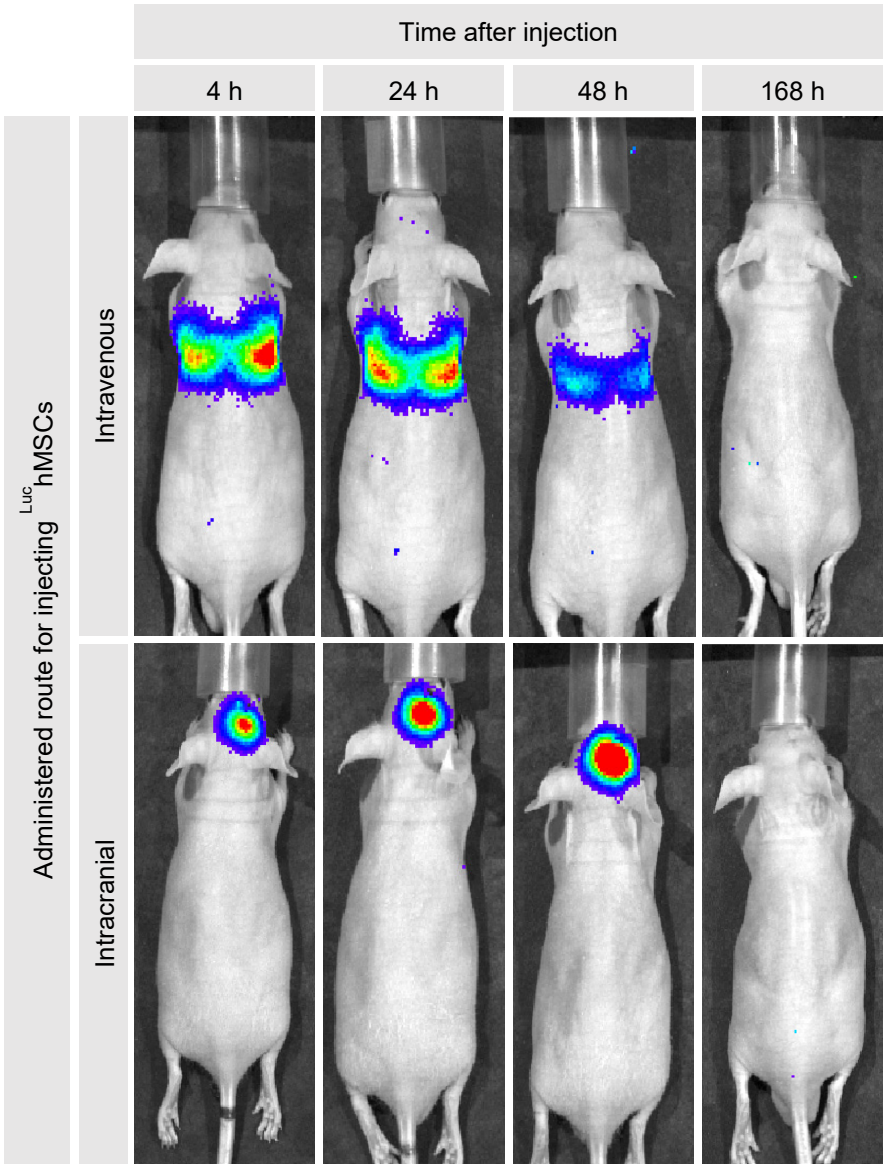

Figure S9. Biodistribution of [luc]hMSCs in nude mice after intravenous or intracranial injection by IVIS. [luc]hMSCs were injected intravenously or intracranially into U87MG-bearing nude mice. The in vivo bioluminescent images were acquired at 4, 24, 48 and 168 hours after injections.