Supporting Information for

Multi-functional Nanotracers for Image-guided Stem Cell Gene Therapy

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Experimental section

Subcellular fractionation: Nuclear and cytoplasmic fractions were isolated using a NE-PER® Nuclear and Cytoplasmic Extraction Reagent Kit (Thermo Scientific, cat.# 78833) as previously described.[1, 2] Briefly, hMSCs were washed with PBS and resuspended in cytoplasmic extract buffer (10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.3% NP-40, and a protease inhibitor cocktail). After gentle rotation at 4°C for 10 min, samples were centrifuged at 3000 rpm for 5 min. Supernatants (cytosolic fraction) were transferred to fresh tubes and stored on ice. Pellets (nuclear fraction) were washed with nuclear extract buffer (20 mM HEPES pH 7.9, 0.4 M NaCl, 1 mM EDTA, 25% glycerol, and a protease inhibitor cocktail), resuspended in PBS, and physically sheared by sonication. Western blot analysis was performed to evaluate the EGFP expression level in each fraction.

References for Supporting Information

1. N.-A. Liu, J. Sun, K. Kono, Y. Horikoshi, T. Ikura, X. Tong, T. Haraguchi and S. Tashiro, *FASEB J.*, 2015, **29**, 2514-2525.
2. H. Shima, H. Suzuki, J. Sun, K. Kono, L. Shi, A. Kinomura, Y. Horikoshi, T. Ikura, M. Ikura and R. Kanaar, *J. Cell. Sci.*, 2013, **126**, 5284-5292.

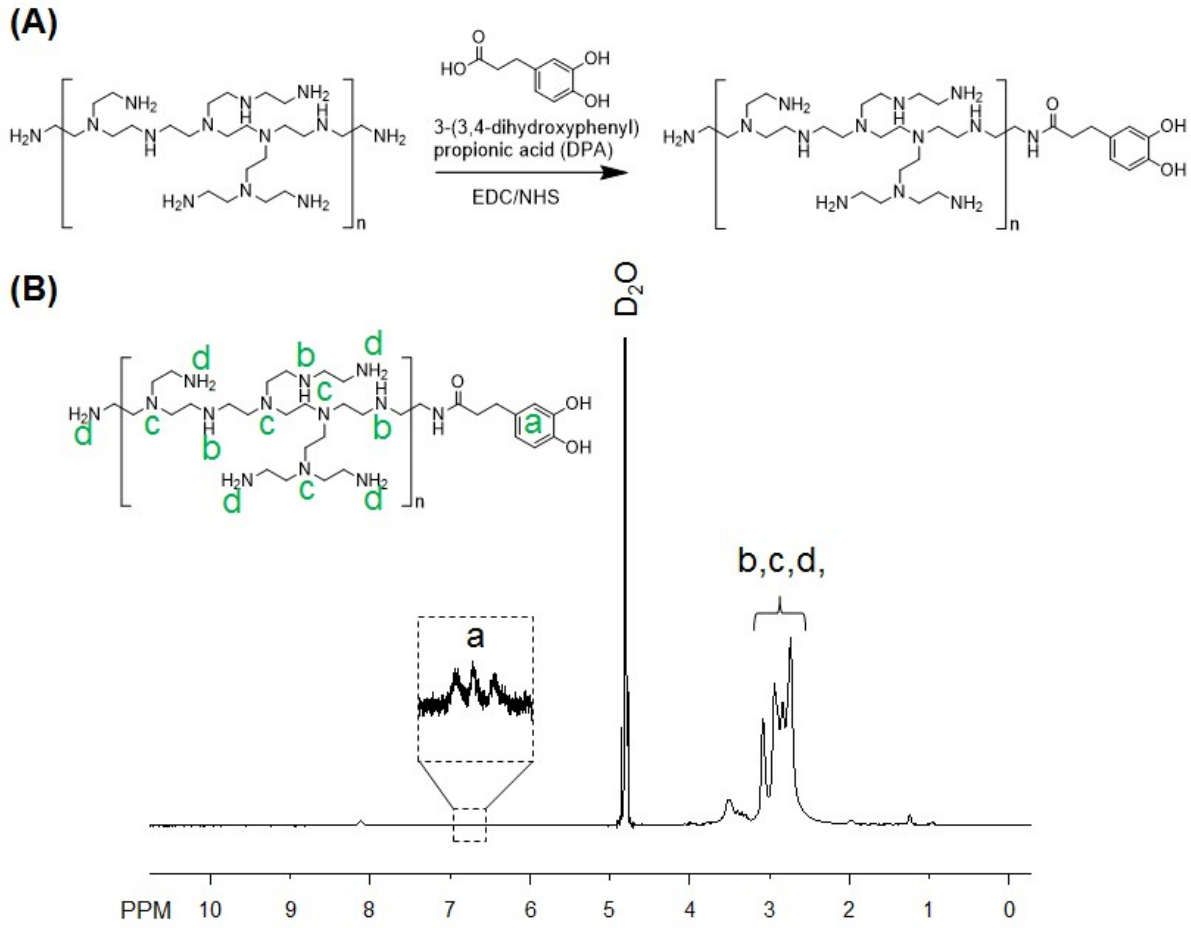

Fig. S1 (A) Synthetic route of C-bPEI. (B) ^{1}H-NMR analysis of C-bPEI in D_2O.

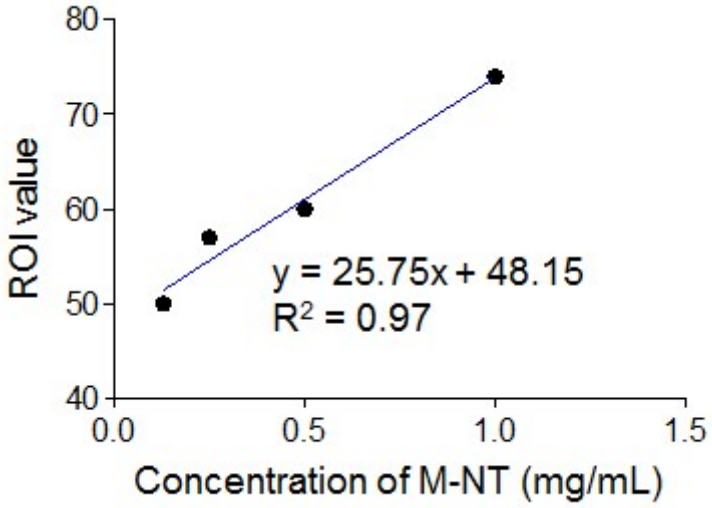

Fig. S2 A linear regression curve obtained by plotting the region of interest (ROI) value of M-NT at different concentration.

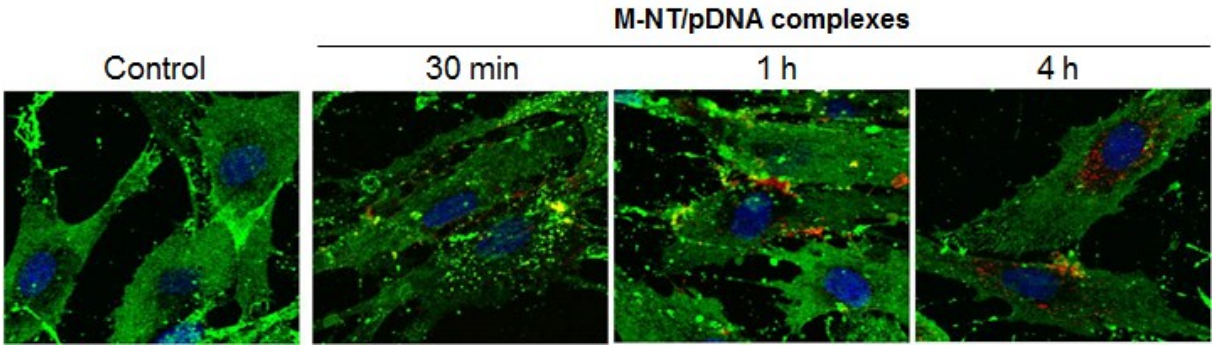

Fig. S3 CLSM images of hMSCs treated with M-NT/pDNA complexes as a function of time (red fluorescence: RITC-labeled M-NTs, green fluorescence: cellular membranes stained with DiO).

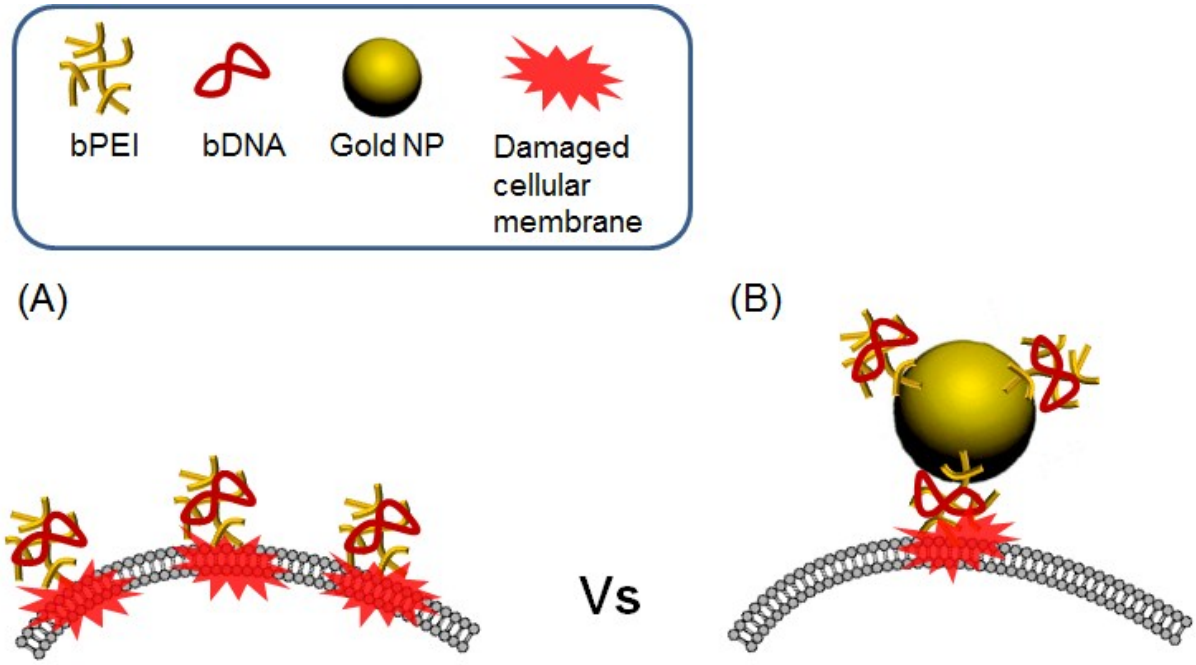

Fig. S4 Schematic illustration of the hypothesized cellular uptake mechanism of bPEI/pDNA versus M-NT/pDNA complexes. When it is assumed that the treated concentrations of bPEI/pDNA and M-NT/pDNA complexes are the same, bPEI/pDNA complexes (A) could have more contact points with the cellular membrane than M-NT/pDNA complexes (B), resulting in more severe cellular membrane damage.

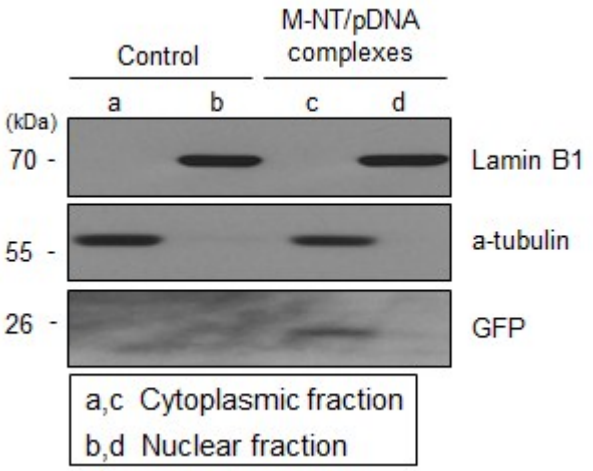

Fig. S5 Western blot analysis of GFP expression in cytoplasmic and nuclear fractions of hMSCs treated with M-NT/pDNA complexes (a-tubulin and lamin B1 are representative protein markers expressed in the cytoplasmic and nuclear fractions, respectively).

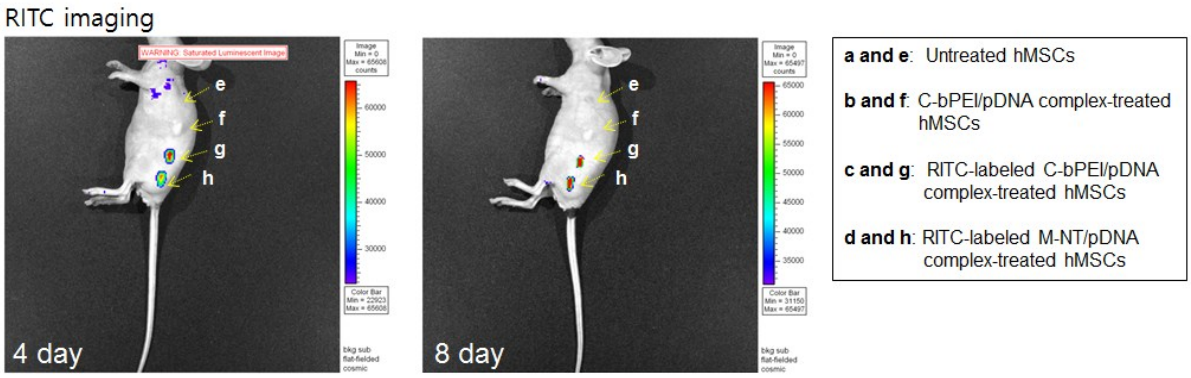

Fig. S6 *In vivo* optical fluorescence of transplanted hMSCs in Balb/c nude mice: RITC images of transplanted hMSCs (treated with M-NT/pDNA complexes) in Balb/c nude mice at 4 and 8 days after transplantation, respectively.

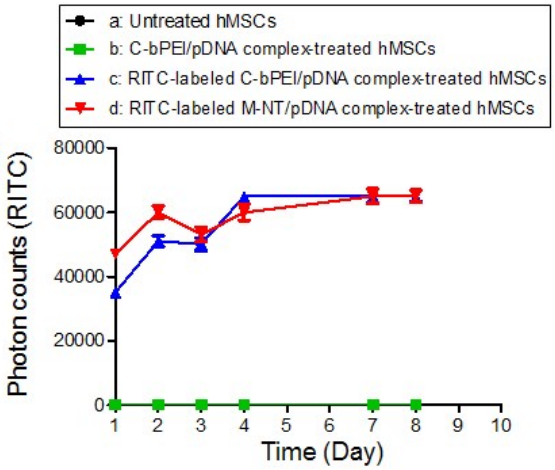

Fig. S7 Quantitative analysis of RITC fluorescence images from hMSCs (untreated or treated with different samples) transplanted into Balb/c nude mice.

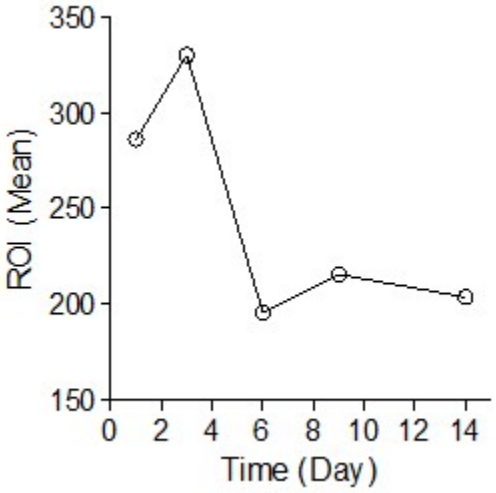

Fig. S8 Quantitative analysis of micro-CT images from hMSCs (treated with M-NT/pEGFP) transplanted into Balb/c nude mice.