Supporting Information

Simple methods for tracking stem cells with ^{64}Cu-labeled DOTA-hexadecyl-benzoate

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Experimental procedures

Synthesis

4-[(9H-Fluoren-9-ylmethoxycarbonylamino)-methyl]-benzoic acid (**3**)

10% $NaHCO_3$ solution (60 mL) was added to a solution of p-Amino methyl benzoic acid (2.03 g, 13.4 mmol) and Fmoc-OSu (4.98 g, 14.77 mmol) in THF (60 mL). The reaction mixture was stirred at room temperature for 3.8 h. The THF was removed and ethyl acetate was added to reaction mixture. The solution was acidified with 1 N HCl solution. The reaction mixture was filtered and alternately washed with ethyl acetate and water. The solid was dried under vacuum to give 2 (4.68 g, 93.5%) as white solid.

4-[(9H-Fluoren-9-ylmethoxycarbonylamino)-methyl]-benzoic acid hexadecyl ester (**4**)

1-Hexadecanol (1.95 g, 8.03 mmol) and PPh3 (2.81 g, 10. 7 mmol) were dissolved in THF at 0 ℃. DIAD (2.12 mL, 10.7 mmol) was added dropwise at same temperature. The reaction mixture was stirred at room temperature for 1 h under an argon atmosphere. Compound **2** (2 g, 5.36 mmol) was added to reaction mixture and stirred overnight at room temperature. The sat. $NaHCO_3$ solution was added and the THF was removed. The reaction mixture was extracted three times with ethyl acetate. The solution was passed through a layer of anhydrous Na_2SO_4 to remove water and concentrated. The crude product was purified by column chromatography (10% ~ 25% EtOAc/Hex) and concentrated. The solid product was washed with hexane, concentrated and dried under vacuum to give 3 (1.96 g, 61,2%) as white solid: ^{1}H NMR (400 MHz, CDCl3); δ 8.01-7.99 (d, 2 H, J = 8 Hz), 7.78-7.76 (d, 2 H, J = 7.6 Hz), 7.61-7.59 (d, 2 H. J = 7.6 Hz), 7.43-7.39 (t, 3 H, J = 7.2 Hz), 7.33-7.30 (t, 3 H, J = 7.4 Hz), 4.50-4.49 (d, 2 H, J = 6.4 Hz), 4.44-4.42 (d, 2 H, J = 6 Hz), 4.33-4.30 (t, 2 H, J = 6.6 Hz), 4.24-4.21 (t, 1 H, J = 6.6 Hz), 1.8-1.73 (p, 2 H, J = 6.4, 7.2 X 2, 6.8 Hz), 1.44-1.21 (m, 28 H), 0.9-0.87 (t, 3 H, J = 6.8 Hz); ^{13}C NMR (100 MHz, CDCl3); δ 156.37, 156.42, 143.78, 143.45, 141.32, 129.92, 129.69, 127.68, 127.15, 127.02, 124.93, 119.97, 66.65, 65.16, 47.26, 44.68, 31.90, 29.67, 29.65, 29.64, 29.58, 29.53, 29.34, 29.28, 28.69, 26.02, 22.68, 14.11, -0.03

Electrospray ionization mass spectrometry (ESI-MS); [M+K+] = 636.48

4-Aminomethyl-benzoic acid hexadecyl ester (**5**)

Piperidine (2.3 mL) was added to a solution of compound 3 (1.12 g, 1.87 mmol) in DCM (6 mL) under an argon atmosphere. After stirring at room temperature for 30 min, the solvent was removed. The hexane was added to the reaction mixture and filtered. The solid product was dried under vacuum to give 4 (600 mg, 85.2%) as white solid: ^{1}H NMR (400 MHz, DMSO-d6); δ 7.91-7.89 (d, 2 H, J = 8.4 Hz), 7.50-7.48 (d, 2 H, J = 8 Hz), 4.26-4.23 (t, 2 H, J = 6.4 Hz), 3.82 (s, 2 H), 1.72-1.66 (p, 2 H, J = 6.8 X 2, 7.2, 6.4 Hz), 1.38-1.23 (m, 28 H), 0.86-0.83 (t, 3 H, J = 6.4, 6.8 Hz); ^{13}C NMR (100 MHz, CDCl3); δ 166.58, 148.28, 129.82, 129.05, 126.86, 65.07, 46.20, 31.91, 29.68, 29.66, 29.64, 29.58, 29.52, 29.35, 29.28, 28.71

ESI-MS; [M+H+] = 376.4

4-{[2-(4,7,10-Tris-tert-butoxycarbonylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetylamino]-methyl}-benzoic acid hexadecyl ester (**6**)

1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (79 uL, 0.528 mmoL) was added to a solution of compound **4** (119.2 mg, 0.317 mmol) in $CHCl_3$ (8 mL). The reaction mixture was stirred at room temperature for 30 min under an argon atmosphere. DOTA-mono-NHS-tris(tBu ester) (215.7 mg, 0.264 mmol) in $CHCl_3$ (5 mL) was added to the solution. The reaction mixture was stirred overnight at room temperature and extracted with DCM and sat. $NaHCO_3$ solution. The organic layer was washed with water. The solution was passed through a layer of anhydrous Na_2SO_4 to remove water and concentrated. The crude product was purified by column chromatography (3 ~ 5% MeOH/DCM) and concentrated. The product was dried under vacuum to give 5 (135.3 mg, 55%) as colorless oil: ^{1}H NMR (400 MHz, CDCl3); δ 7.94-7.92 (d, 2 H, J = 8.4 Hz), 7.38-7.36 (d, 2 H, J = 8.4 Hz), 6.86-6.83 (t, 1 H, J = 6.4, 6 Hz), 4.46-4.44 (br, 2 H), 4.3-4.26 (t, 2 H, J = 6.4, 6.8 Hz), 3.33 (br, 4 H), 2.91 (br, 7 H), 2.54 (br, 4 H), 2.22 (br, 7 H), 1.78-1.71 (p, 2 H, J = 6.8 X 2, 8, 6.8 Hz), 1.46 (s, 9 H), 1.39 (s, 18 H), 1.34-1.25 (m, 28 H), 0.89-0.86 (t, 3 H, J = 6.8, 7.2 Hz); ^{13}C NMR (100 MHz, CDCl3); δ 172.49, 171.96, 166.60, 144.33, 129.64, 128.98, 127.65, 81.80, 81.76, 77.20, 64.96, 55.81, 55.62, 42.88, 31.89, 29.67, 29.62, 29.60, 29.55, 29.32, 29.30, 28.73, 27.87, 26.02, 22.66

ESI-MS; [M+Na+] = 953.0

4-{[2-(4,7,10-Tris-carboxymethyl-1,4,7,10tetraaza-cyclododec-1-yl)-acetylamino]-methyl}-benzoic acid hexadecyl ester (**2**)

Compound **5** (131.4 mg, 0.141 mmol) in trifluoroacetic acid (TFA):DCM:Et_3SiH = 8:2:1 solution (4.4 mL) was stirred at room temperature for 24 h. The product was confirmed with ESI-MS. The particles were removed by Buchner funnel. The solution was concentrated and added ether. The solution was concentrated and added ether. The solid was filtered and the solvent was removed to give 1 (102 mg, 59%) as a white solid**:** ^{1}H NMR (400 MHz, CD_3OD); δ 7.97-7.95 (d, 2 H, *J* = 8.4 Hz), 7.43-7.41 (d, 2 H, *J* = 8.0 Hz), 4.43 (s, 2 H), 4.31-4.28 (t, 2 H, *J* = 6.4, 6.8 Hz), 3.86-3.12 (br, 24 H), 1.79-1.72 (p, 2 H, *J* = 6.8 X 2, 8, 6.8 Hz), 1.46-1.11 (m, 26 H), 0.91-0.87 (t, 3 H, *J* = 6.8, 7.2 Hz); ^{13}C NMR (100 MHz, CD_3OD); δ167.94, 163.36, 163.02, 162.68, 162.33, 145.35, 130.75, 130.42, 128.79, 122.55, 119.63, 116.70, 113.78, 106.39, 66.22, 55.85, 55.04, 51.10, 43.84, 33.07, 30.79, 30.77, 30.73, 30.69, 30.48, 30.42, 29.81, 27.15, 23.74, 14.50
ESI-MS; [M+H+] = 762.8
HRMS data :calculated : 761.49, found : 762.4983

Primary ADSCs culture

The care, maintenance, and treatment of animals in these studies followed protocols approved by the Institutional Animal Care and Use Committee of the Korea Institute of Radiological and Medical Sciences (KIRAMS). Male Sprague-Dawley (SD) rats (Narabio, Seoul, Korea) weighing 250 ± 10 g were used. ADSCs were isolated from SD rats euthanized by carbon dioxide (CO_2) inhalation. Visceral fat encasing the stomach and intestine was aseptically dissected and minced to 1–3 mm with a sterile surgical blade. The isolated tissue was enzymatically dissociated for 15 min at 37℃ using 0.1% (w/v) collagenase type I (Worthington Biochemical Corp., Lakewood, NJ). Undissociated tissue was removed by filtering using 70 μm nylon mesh, then neutralized using Dulbecco's Modified Eagle's Medium (DMEM) with 10% (v/v) fetal bovine serum (FBS), and centrifuged at 250 × g for 5 min. The cell pellet was resuspended in DMEM containing 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin solution. Cultures were maintained in a 37℃ incubator with 5% CO_2, and the medium was changed every 3 days.

^{64}Cu labeling with 2 and cell labeling with ^{64}Cu-DOTA-HB (1)

^{64}Cu was produced at KIRAMS by 50 MeV cyclotron irradiation using methods reported.[1] Briefly, 22.3μL of 2 (1mg/mL in 1M acetic acid solution) was added to 1mL of ^{64}Cu-acetic acid buffer solution. The radioactive fraction was checked for radiochemical purity by silica gel radio-thin layer chromatography (radio-TLC) using 20 Mm citric acid/50 mM EDTA solution as a mobile phase. A solution of 1 (37–74 MBq, 200 μL) in 20% dimethyl sulfoxide (DMSO)/phosphate buffered saline (PBS) was added to 3 × 10^6 rat ADSCs in 2 mL PBS, and the mixture was incubated at 37 ℃ for 1 h. After centrifugation (250 × g, 5 min), the supernatant was removed, and the cells were washed twice with PBS solution. The radioactive content of the isolated pellet and supernatant was measured to calculate radiolabeling efficiency using a radioisotope calibrator (CRC® -127R; Capintec, Inc., Ramsey, NJ). This procedure was repeated three times to ensure accurate and efficient cell labeling.

***In vivo* small animal positron emission tomography (PET) imaging**

The images of mice were obtained using a small animal PET/computed tomography (CT) scanner (Inveon™; Siemens Preclinical Solutions, Malvern, PA). Animals were anesthetized with 2% isoflurane and injected with 2.8 – 10.0 MBq (100 μL) of 1 of via lateral tail vein injection, and 30-min static scans were acquired at 1 h after injection. CT images were acquired at 70 kVp of X-ray voltage with a 400 μA anode current with a 200 msec exposure time for each step. PET emission data were acquired with three spans and 79 ring differences through a 350–650 keV energy window and 3.43 nsec timing windows. PET images were reconstructed using an ordered subset expectation maximization (OSEM) 2D algorithm with 4 iterations. Regions of interest (ROIs) were drawn on the reconstructed images using Inveon Research Workplace [(IRW), provided by Siemens Preclinical Solutions].

Digital whole-body autoradiography

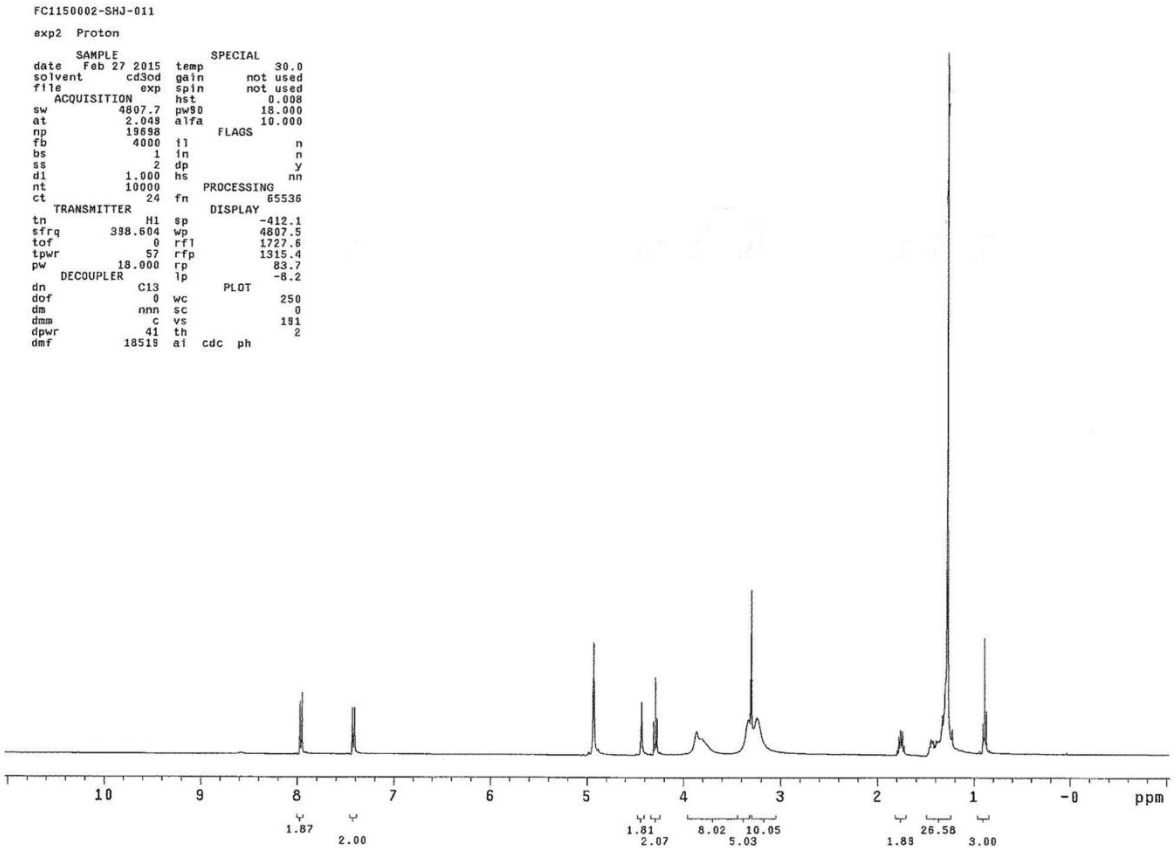
One hour after 1 intravenous injection, mice were euthanized by a CO_2 inhalation. The digital whole-body autoradiography was performed by previously described methods.[2] Briefly, the sacrificed mice froze at -70℃ deep freezer. Coronal whole-body mouse sections (30-μm thick) were obtained by whole-body autocryotome (Nakagawa Seisakusho Co., Ltd., Tokyo, Japan), the frozen sections were exposed to an image plate for 1 hour, and the plates were scanned with BAS-5000 (Fujifilm, Tokyo, Japan). An autoradiogram and its matched photo image were shown to the adjacent of the small-animal PET image to link the radioactive signal with anatomy.

Statistical analysis

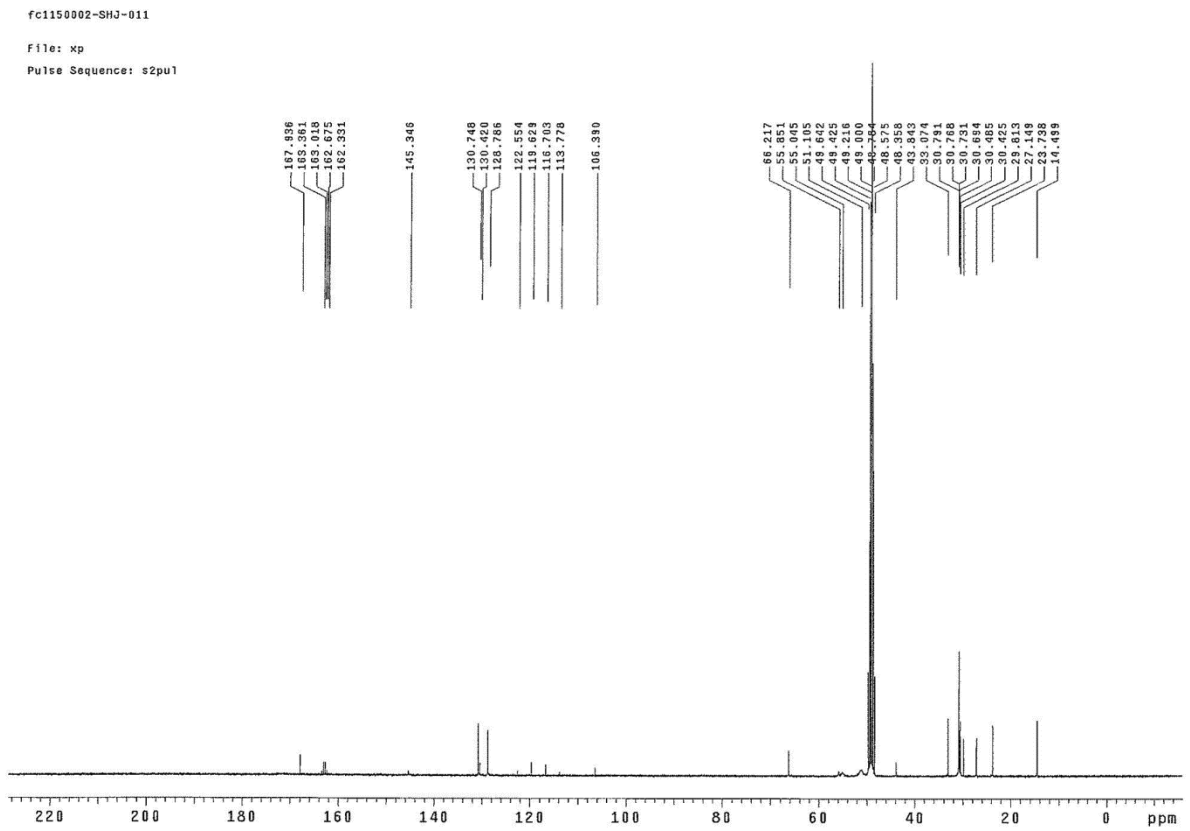
All the data were expressed as mean ± standard deviation (SD), and were analyzed using statistical software

(GraphPad Prism Inc., La Jolla, CA) Student's *t*-test was performed to compare mean differences between groups. All statistical analyses were considered as significant if the *P*-value was less than 0.05.

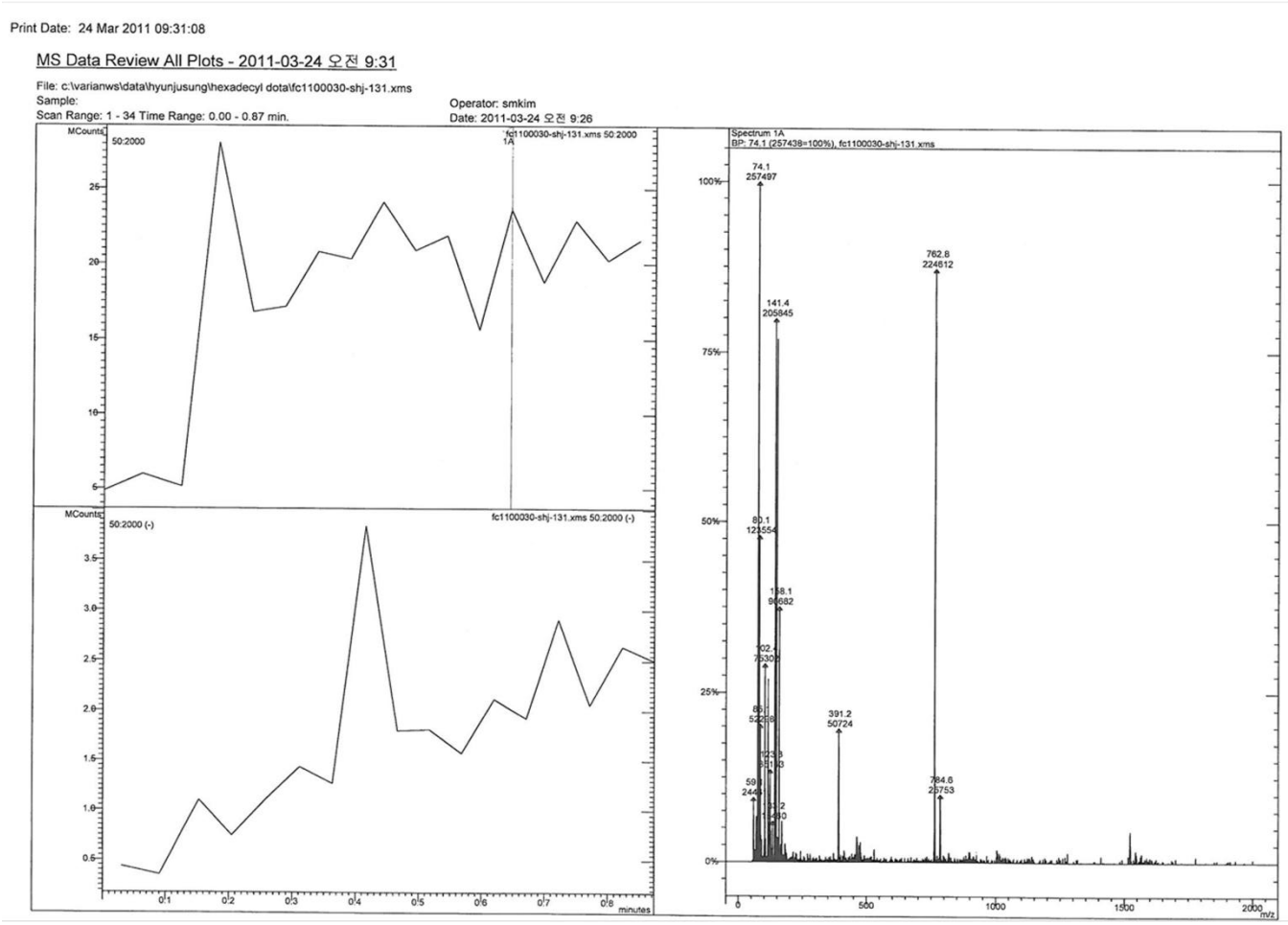
Supplementary Figures



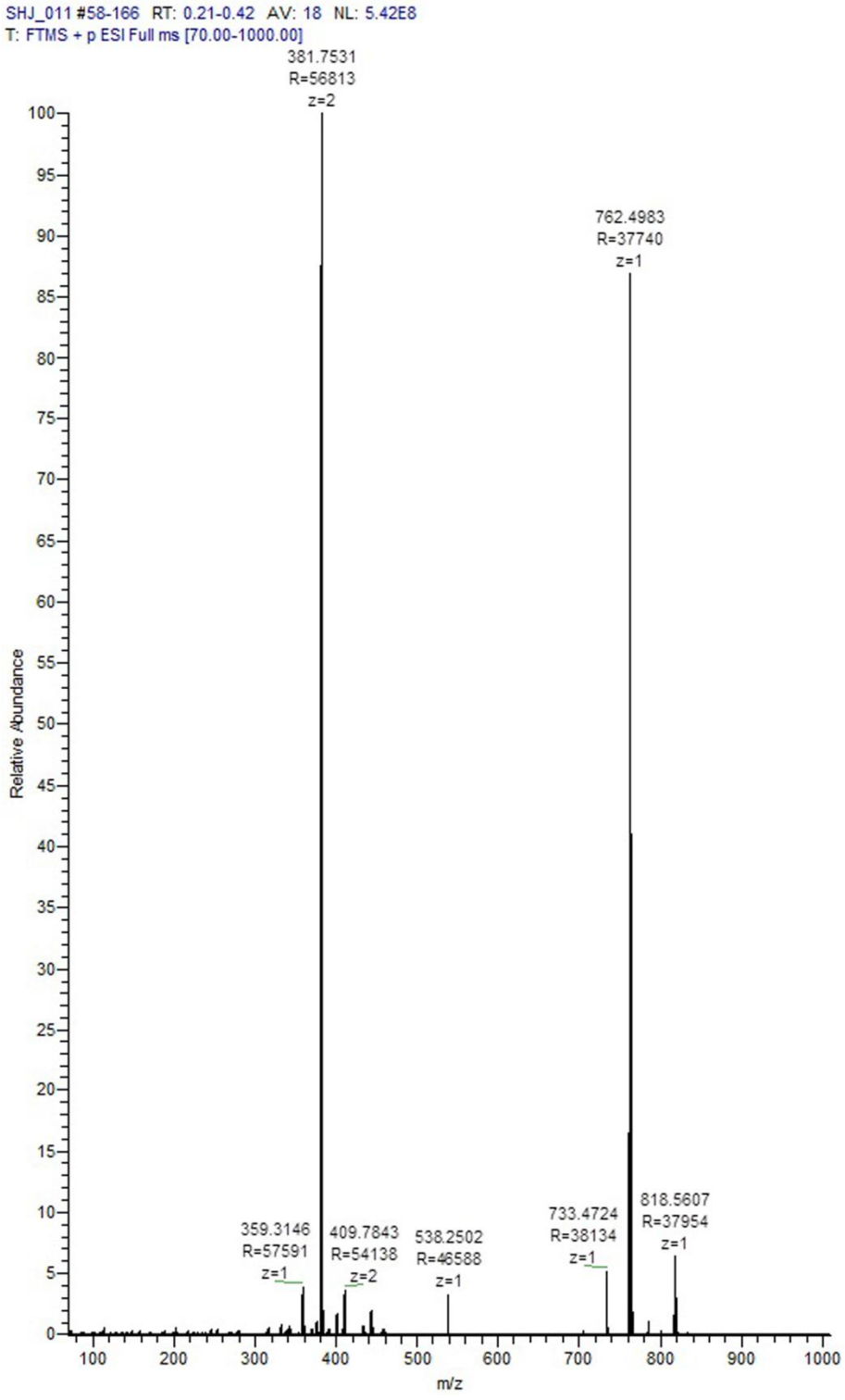
Supplementary Figure 1S. ^{1}H nuclear magnetic resonance (NMR) analysis of **2**.



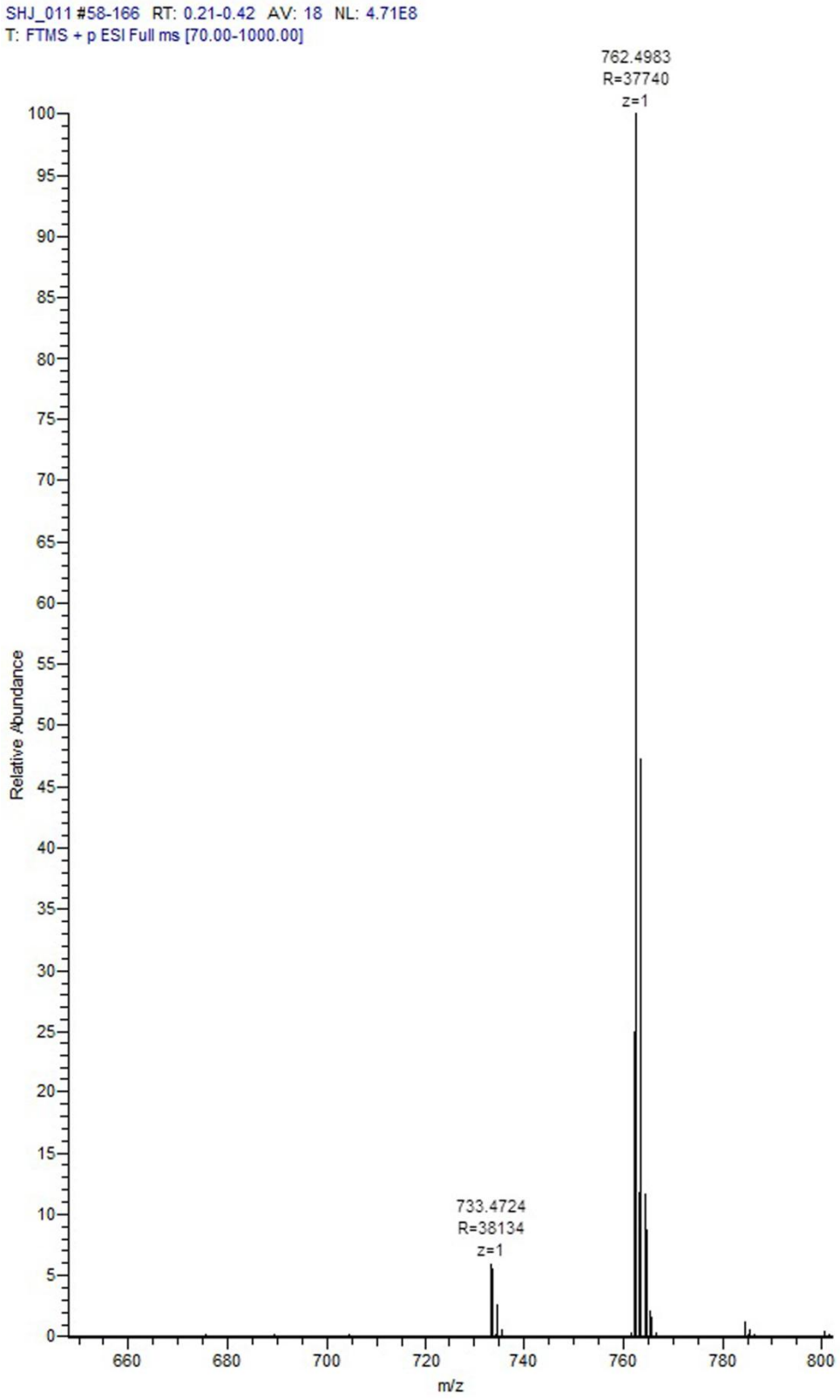
Supplementary Figure 2S. ^{13}C NMR analysis of **2**.



Supplementary Figure 3S. Electrospray ionisation mass spectrometry (ESI-MS) of compound **2**.



Supplementary Figure 4S. High resolution mass spectrometer (HRMS) analysis of **2** with values ranging from 100-1000 (mass /charge (m/z)).

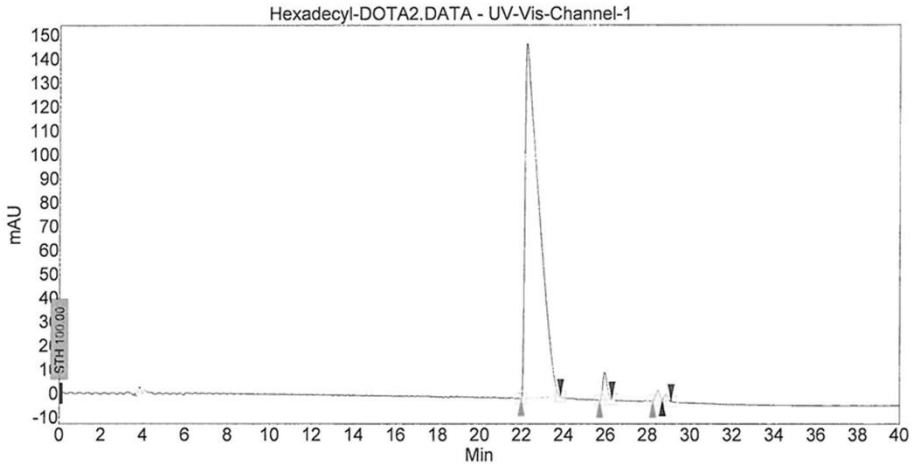


Supplementary Figure 5S. HRMS analysis of **2** with values ranging from 660-800 (m/z).

Chromatogram : Hexadecyl-DOTA2_channel1

System : 410_440
Method : 50to100%Bfor30min-auto410440
User : Hyunju Sung

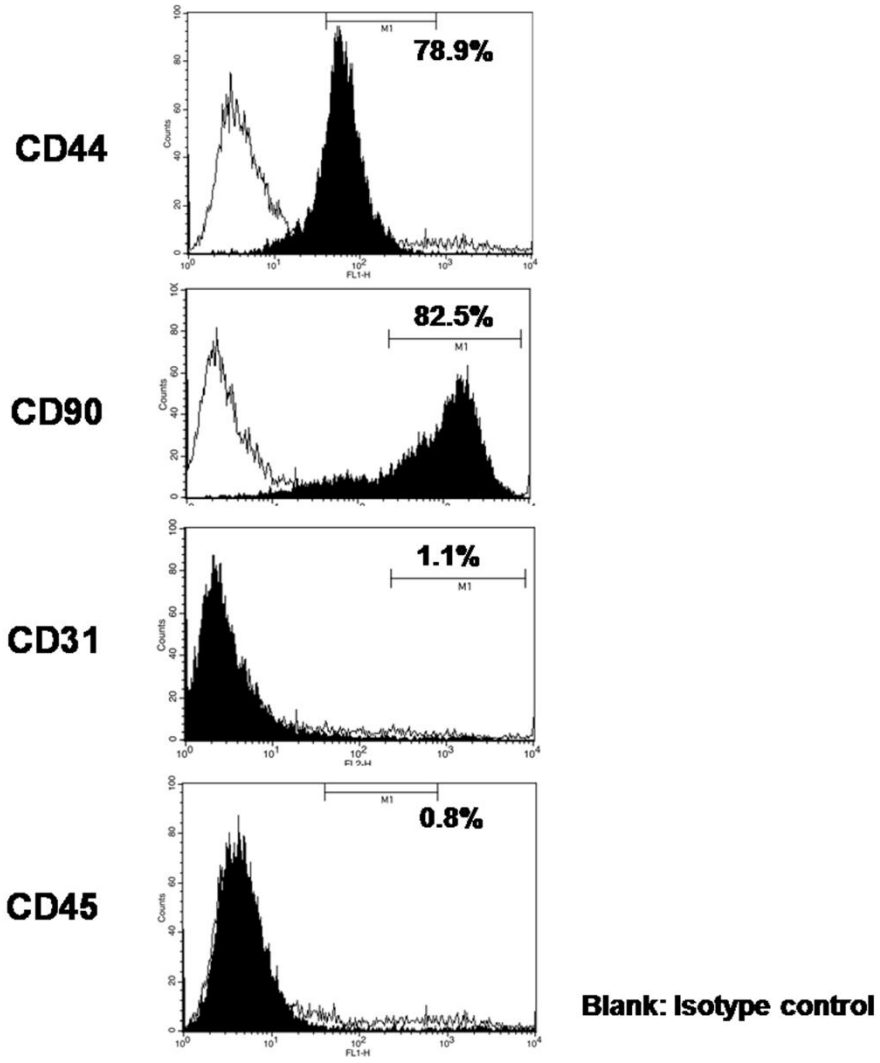
Acquired : 3/28/2011 11:21:38 AM
Processed : 3/28/2011 1:06:05 PM
Printed : 3/28/2011 1:07:08 PM



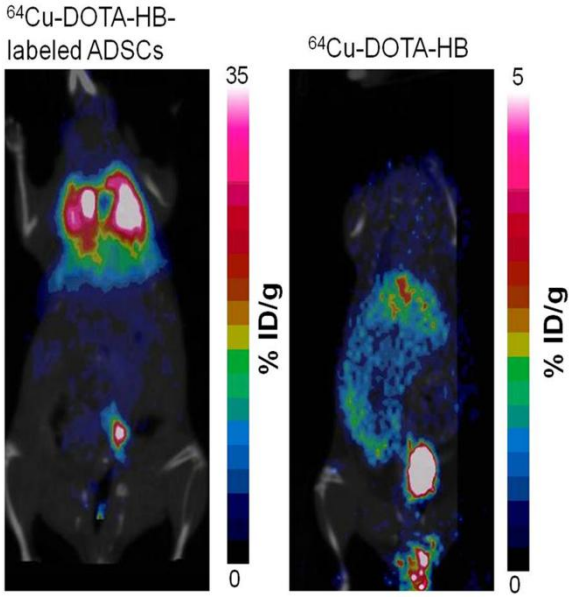
Peak results :

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU Min]	Area % [%]
1	UNKNOWN	22.22	96.31	148.4	112.9	96.306
2	UNKNOWN	25.95	2.31	11.5	2.7	2.308
3	UNKNOWN	28.49	0.84	4.3	1.0	0.843
4	UNKNOWN	28.87	0.54	3.0	0.6	0.543
Total			100.00	167.1	117.3	100.000

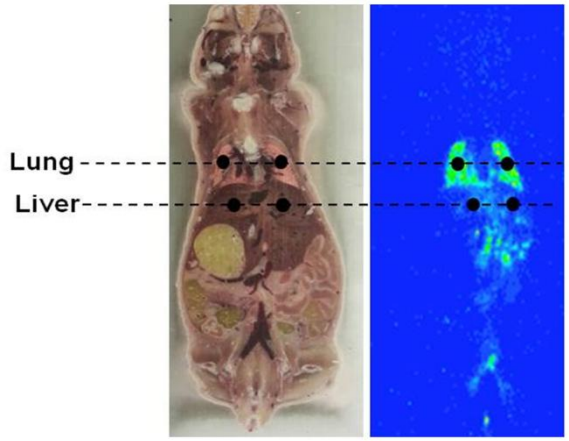
Supplementary Figure 6S. High performance liquid chromatography (HPLC) analysis of compound **2**.



Supplementary Figure 7S. Flow cytomeric analysis of ADSCs.



Supplementary Figure 8S. Small animal PET/CT image of nude mice after 1h intravenous injection of **1**-labeled ADSCs (2.8 – 10.0 MBq, 100 μL) (left) and ^{64}Cu-DOTA-HB (right).



Supplementary Figure 9S. Photo image of coronal whole-body mouse section (left) and digital whole body autoradiography (right) of mice intravenously injected with **1**-labeled ADSCs (2.8 – 10.0 MBq, 100 µL) at 1 h post-injection.

Supplementary references

(1) Kim, J. Y.; Park, H.; Lee, J. C.; Kim, K. M.; Lee, K. C.; Ha, H. J.; Choi, T. H.; An, G. I.; Cheon, G.J.; A simple Cu-64 production and its application of Cu-64 ATSM. **2009**, *Appl. Radiat. Isot.* 67, 1190−1194.

(2) Gambhir, S. S.; Barrio, J. R.; Wu, L.; Iyer, M.; Namavari, M.; Satyamurthy, N.; Bauer, E.; Parrish, C.; MacLaren, D. C.; Borghei, A. R.; Green, L. A.; Sharfstein, S.; Berk, A. J.; Cherry, S. R.; Phelps, M. E.; Herschman, H. R. Imaging of adenoviral-directed herpes simplex virus type 1 thymidine kinase reporter gene expression in mice with radiolabeled ganciclovir. **1998**, *J. Nucl. Med.* 39, 2003−2011.