Supplemental Material

MATERIALS AND METHODS

General.

HPLC Method 1: Phenomenex® C18 column (250 × 4.6mm, 5 micron); 1 mL/min flow rate; and eluent gradient: 0–2 min 95% solvent A [0.1% trifluoroacetic acid (TFA) in water] and 5% solvent B [0.1% TFA in acetonitrile (MeCN)]; 2-7 min 95-85% solvent A and 5-15% solvent B; 7-27 min 85-70% solvent A and 15-30% solvent B. HPLC Method 2: Phenomenex® C18 column (250 × 4.6mm, 5 micron); 1 mL/min flow rate; and eluent gradient: 0−2 min 95% solvent A and 5%solvent B; 2−22 min 95-5% solvent A and 5−95% solvent B. HPLC Method 3: Phenomenex® C18 column (250 × 4.6mm, 5 micron); 1 mL/min flow rate; and eluted with a stepwise gradient: 0−2 min, 95% solvent A and 5% solvent B; 2−32 min 95-65% solvent A and 5−35% solvent B.

All ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz (Varian), at room temperature. Spectra were obtained on $CDCl_3$, D_2O solutions in 5 mm diameter tubes, and the chemical shift in ppm is quoted relative to the residual signals of $CDCl_3$ (δH 7.26 ppm, or δC 77.2 ppm) or D_2O (δH 4.65 ppm). Multiplicities in the ^{1}H NMR spectra are described as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. All products were characterized by mass spectrometry on a Thermo Electron LTQ-FT mass spectrometer.

Chemistry.

*Synthesis of 2-(2-hydroxyethoxy)ethyl 4-nitrobenzenesulfonate (**1**).* 4-Nitrobenzene sulfonyl chloride (12.5 g, 56.6 mmol) was added to a solution of diethylene glycol (5 g, 47 mmol) and triethylamine (9 ml, 66 mmol) in 150 mL of dichloromethane (Fig. 1A). The reaction mixture was stirred at room temperature overnight, and washed with water and brine. After evaporation of solvent, the residue was purified by column chromatography (dichloromethane/ether, 9:1) to give **1** (yield: 25~30%) as colorless solid. The product was characterized by mass spectrometry (m/z 292.05 for $[MH]^+$, $C_{10}H_{14}NO_7S$, calculated $[MH]^+$ 292.04). ^{1}H-NMR($CDCl_3$): 8.42(d, J=8.8Hz,2H); 8.15(d ,J=8.8Hz,2H); 4.34(bt, J=5.2Hz,2H); 3.75(bt, J=5.2Hz,2H); 3.70(t

,J=5.2Hz,2H); 5.56(t ,J=5.2Hz,2H). ^{13}C-NMR ($CDCl_3$): 150.5(s), 141.9(s), 129.3(dX2), 124.5(dX2), 72.6(t), 70.3(t), 68.5(t), 61.7(t).

*Synthesis of 2-(2-(2-(vinylsulfonyl)ethoxy)ethoxy)ethyl 4-nitrobenzenesulfonate (**2**).* *t*-BuOK (50 uL, 50% in MeOH) was added to a solution of **1** (1 g, 3.4 mmol) and divinylsulfone (2.2 mL, 20 mmol). The reaction mixture was stirred at room temperature for 4 hr. The reaction mixture was purified by column chromatography (dichloromethane/ether, 9:1) to give **2** (yield: 70~80%) as a colorless solid. The product was characterized by mass spectrometry (m/z 432.04 for $[MNa]^+$, $C_{14}H_{19}NNaO_9S_2$, calculated $[MNa]^+$ 432.04). ^{1}H-NMR($CDCl_3$): 8.41(d, J=8.8Hz,2H); 8.13(d, J=8.8Hz,2H); 6.78(dd, J=16.6Hz,10.0Hz,1H); 6.41(d, J=16.6Hz,1H); 6.11(d, J=10.0Hz,1H); 4.29(bt, J=5.1Hz,2H); 3.88(t, J=6.2Hz,2H); 3.72(t, J=5.1Hz,2H); 3.58(s,2HX2); 3.24(t, J=6.2Hz,2H). ^{13}C-NMR($CDCl_3$): 150.8(S), 141.8(S), 137.9(d), 129.3(dX2), 129.0(t), 124.5(dX2), 70.4(tX3), 68.5(t), 64.6(t), 54.9(t).

*Synthesis of (2-(2-(2-fluoroethoxy)ethoxy)ethylsulfonyl)ethene (^{19}F-DEG-VS, **3**).* **2** (12 mg, 41.2 μmol) in 50 μL DMSO was added 30 μL TBAF (1M TBAF in THF) and reacted at 78 °C overnight. The reaction mixture was purified on a C18 column on HPLC to give 2.8 mg **3** (yield: 41.3%) as a yellowish liquid. The product was characterized by mass spectrometry (m/z 227.0746 for $[MH]^+$, $C_8H_{15}FO_4S$, calculated $[MH]^+$ 227.0748). ^{1}H-NMR(D_2O): 6.79(dd, J=16.5Hz,10.1Hz,1H), 6.32(d, J=16.5Hz, 1H), 6.20(d, J=10.1Hz, 1H), 4.51(dt,$^2J_{H,F}$=47.8Hz,J=4.1Hz,2H), 3.84(t, J=5.6Hz, 2H), 3.69(dt,$^3J_{H,F}$=31.9Hz,J=4.1Hz, 2H), 3.60(m, 4H), 3.42(t, J=5.6Hz, 2H).

Synthesis of ^{19}F-DEG-VS-c(RGDyC). *c*(RGDyC) (1mg, 1.68 μmol, 100 μL H_2O) and ^{19}F-DEG-VS (500 μg, 2.2 μmol, 5 μL MeCN and 45 μL H_2O) were added into 900 μL of phosphate buffer (pH 7.0). The reaction mixture was incubated at room temperature for 30 min and was purified by HPLC using Method 1. The retention time of ^{19}F-DEG-VS-*c*(RGDyC) was 17.2 min. The final product was a white solid after lyophilization. The product was characterized by mass spectrometry (m/z 821.29 for $[MH]^+$, $C_{32}H_{49}FN_8O_{12}S_2$, calculated $[MH]^+$ 821.29).

Synthesis of ^{19}F-DEG-VS-c(RGDyK). *c*(RGDyK) (1mg, 1.61 μmol, 100 μL H_2O) and ^{19}F-DEG-VS (500 μg, 2.2 μmol, 5 μL MeCN and 45 μL H_2O) were added into 900 μL of phosphate buffer (pH 9.0). The reaction mixture was incubated at room temperature overnight and was purified by HPLC using Method 1. The retention time of ^{19}F-DEG-VS-*c*(RGDyK) was 13.1 min. The final product was a white solid after lyophilization. The product was characterized by mass spectrometry (m/z 846.38 for $[MH]^+$, $C_{32}H_{49}FN_8O_{12}S_2$, calculated $[MH]^+$ 846.38).

Synthesis of ^{19}F-DEG-VS-Neurotensin and ^{19}F-DEG-VS-(Ac)-Neurotensin. The neurotensin analog (NT, Cys-pipGly-Pro-pipAmGly-Arg-Pro-Tyr-tBuGly-Leu-OH, 300 μg (0.25 μmol) in 30 μL H_2O) or (Ac)-NT and ^{19}F-DEG-VS (500 μg, 2.2 μmol, 5 μL MeCN and 45 μLH_2O) were added into 900 μL of 0.1M borate buffer (pH 8.5). The reaction mixture was incubated at RT for 30 min and purified by HPLC using Method 1. The retention time of ^{19}F-DEG-VS-NT and (^{19}F-DEG-VS)$_2$-NT was 20.08 min and 23.09 min respectively. The final products were white solids after lyophilization. The products were characterized by mass spectrometry (^{19}F-DEG-VS-NT: m/z 1,409.75 for $[MH]^+$, $C_{63}H_{105}FN_{16}O_{15}S_2$, calculated $[MH]^+$ 1,409.74; (^{19}F-DEG-VS)$_2$-NT: m/z 1,635.82 for $[MH]^+$, $C_{71}H_{120}F_2N_{16}O_{19}S_3$, calculated $[MH]^+$ 1,635.80). The retention time of ^{19}F-DEG-VS-(Ac)-NT was 28.18 min on radio-HPLC. The final product was confirmed by mass spectrometry (^{19}F-DEG-VS-(Ac)-NT: m/z 1,451.76 for $[MH]^+$, $C_{65}H_{108}FN_{16}O_{16}S_2$, calculated $[MH]^+$ 1,451.75.

Synthesis of ^{19}F-FBEM-c(RGDyC). FBEM was synthesized as previously described (*12*). FBEM (420μg, 1.6 μmol, 50 μL H_2O) and *c*(RGDyC) (500 μg, 0.8 μmol, 50 μL H_2O) were added into 200 μL PBS (pH 7.5). The reaction mixture was incubated at RT for 30 min and purified by HPLC using Method 3. The retention time of ^{19}F-FBEM-*c*(RGDyC) was 18.2 min. The final product was confirmed by mass spectrometry (^{19}F-FBEM-*c*(RGDyC): m/z 857.30 for $[MH]^+$, $C_{37}H_{45}FN_{10}O_{11}S$, calculated $[MH]^+$ 857.31.

Synthesis of ^{19}F-FBEM-NT. FBEM (130μg, 0.5 μmol, 50 μL H_2O) and NT (300 μg, 0.25 μmol, 50 μL H_2O) were added into 200 μL PBS (pH 7.5). The reaction mixture was incubated at RT for 30

min and purified by HPLC using Method 3. The retention time of ^{19}F-FBEM-NT was 18.9 min. The final product was confirmed by mass spectrometry (^{19}F-FBEM-NT: m/z 1445.74 for $[MH]^+$, $C_{68}H_{101}FN_{18}O_{14}S$, calculated $[MH]^+$ 1445.76).

Radiochemistry.

Synthesis of ^{18}F-(2-(2-(2-fluoroethoxy)ethoxy)ethylsulfonyl)ethane (^{18}F-DEG-VS). ^{18}F-Fluoride (200 mCi) was trapped onto a Sep-Pak QMA cartridge. Tetrabutylammonium bicarbonate (TBAB) solution (4%, 400 μL H_2O) was used to elute the ^{18}F-fluoride from the QMA cartridge into a dried v-vial. The resulting solution was azeotropically dried with sequential MeCN evaporations at 90 °C. A solution of **2** (10 mg in 50 μL of anhydrous DMSO) was added to the reactor and heated at 80 °C for 10 -15 min. Acetic acid (5%, 800 μL) was added to quench the reaction. A portion of the crude mixture (5 mCi) was then purified by radioHPLC using Method 2. The retention time of ^{19}F-DEG-VS was 12.2 min. In order to determine the isolated yield, the reaction crude mixture was loaded onto a semi-prep HPLC using same gradient as method 2 except 4 mL/min flow rate. The product was concentrated using rota-vap.

Synthesis of ^{18}F-DEG-VS-c(RGDyC). ^{18}F-DEG-VS (100 μL, 1 mCi) and *c*(RGDyC) (100 μg, 0.16 μmol) were added into 50 μL of borate buffer (pH 8.5) and the reaction mixture was incubated at room temperature for 5-10 min. The reaction was quenched by acetic acid (5%, 600 μL) and product was purified by radioHPLC Method 1. The retention time of ^{18}F-DEG-VS-*c*(RGDyC) was 21.5 min.

Synthesis of ^{18}F-DEG-VS-c(RGDyK). ^{18}F-DEG-VS (100 μL, 1 mCi) and 100 μL of borate buffer (pH 9.0) were added into *c*(RGDyK) (105 μg, 0.16 μmol) and the reaction mixture was incubated at room temperature for 30 min. The reaction was quenched by acetic acid (5%, 600 μL) and product was analyzed by radioHPLC using Method 1. No product was observed.

Selectivity test: c(RGDyK) and c(RGDyC). ^{18}F-DEG-VS (100 μL, 1 mCi) and 50 μL of borate buffer (pH 8.5) were added into *c*(RGDyC) (100 μg, 0.16 μmol) and *c*(RGDyK) (105 μg, 0.16 μmol). The reaction mixture was incubated at room temperature for 30 min. The reaction was quenched by acetic acid (5%, 600 μL) and product was analyzed by radioHPLC using Method 1.

Synthesis of ^{18}F-DEG-VS-NT and ^{18}F-DEG-VS-(Ac)-NT. ^{18}F-DEG-VS (100 μL, 1 mCi) and 30 μL of borate buffer (pH 8.5) were added into Neurotensin (100 μg, 0.084 μmol) or (Ac)-NT and the reaction mixture was incubated at room temperature for 5-10 min. The reaction was quenched by acetic acid (5%, 600 μL) and product was purified by radioHPLC using Method 1. The retention time of ^{18}F-DEG-VS-NT was 25.6 min and (^{18}F-DEG-VS)$_2$-NT was not observed. In order to study the stability of ^{18}F-DEG-VS-NT, the final product was incubated with 1×PBS for 5 h at 37 °C. An aliquot of the solution was then analyzed by radio-HPLC to determine the radiochemical purify. The retention time of ^{18}F-DEG-VS-(Ac)-NT was 28.4 min.

Synthesis of ^{18}F-FBEM-c(RGDyC). ^{18}F-FBEM (100 μL, 1 mCi) and 30 μL of PBS (pH 7.5) were added into a vial containing 100 μg *c*(RGDyC) (0.16 μmol). The reaction mixture was incubated at room temperature for 15 min. The reaction was quenched by acetic acid solution (5%, 600 μL) and the product was purified by radioHPLC using Method 3. The retention time of ^{18}F-FBEM-*c*(RGDyC) was 18.4 min.

Synthesis of ^{18}F-FBEM-NT. ^{18}F-FBEM (100 μL, 1 mCi) and 30 μL of PBS (pH 7.5) were added into a vial containing 100 μg neurotensin peptide (0.084 μmol). The reaction mixture was incubated at room temperature for 15 min. The reaction was quenched by acetic acid solution (5%, 600 μL) and the product was purified by radioHPLC using Method 3. The retention time of ^{18}F-FBEM-NT was 19.0 min.

***In Vitro* Cell Binding Assay.**

In detail, HT-29 cells were placed in 48-wells plates (1 million/0.4 mL/ well) and were incubated overnight. The cells were washed three times with binding buffer (50 mM Hepes, 125 mM NaCl, 7.5 mM KCl, 5.5 mM $MgCl_2$, 1 mM EGTA, 5 g/L BSA, 2 mg/L chymostatin, 100 mg/L soybean trypsin inhibitor, 50 mg/L bacitracin, pH 7.4). HT-29 cells were incubated in triplicate with 25,000 cpm of ^{125}I-NT(8-13) and variable concentrations (0.001–1,000 nM) of ^{19}F-DEG-VS-NT and NT(8-13) for 1 h at 37°C. After washing, cells were solubilized with 1 mol/L NaOH at 37°C and the radioactivity was determined using a gamma counter. The best-fit 50% inhibitory concentration (IC_{50}) values for HT-29 cells were calculated by fitting the data with nonlinear regression using GraphPad Prism (GraphPad Software). Experiments were performed on triplicate samples.

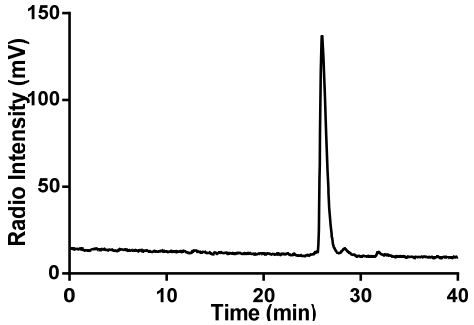
microPET Image reconstruction.

Images were reconstructed with a MAP iterative algorithm using the microPET Manager Software (Concorde Microsystems, Knoxville, TN), and were then analyzed using the Acquisition Sinogram Image Processing (ASIPro) software (Concorde Microsystems, Knoxville, TN). Average radioactivity accumulation within the liver was obtained from a 3-dimensional region of interest (ROI) drawn within the central portion of the PET liver image. Image intensities were converted to units of activity concentration (Bq/cm^3) using a calibration factor obtained by scanning a cylindrical phantom filled with a known activity concentration of ^{18}F. Assuming a tissue density of 1 g/mL, measured tissue activity concentrations were converted to units of Bq/g, then multiplied by 100 and divided by the injected dose to obtain an image-derived percent injected dose per gram of tissue (%ID/g).

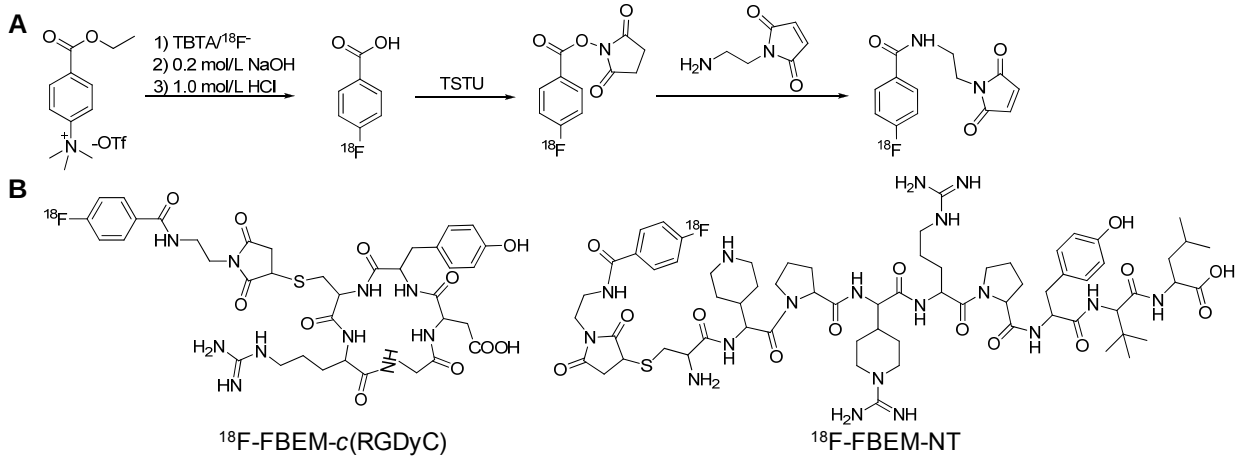
***In Vivo* Metabolic Stability.**

The *in vivo* metabolic stability of ^{18}F-DEG-VS-NT and ^{18}F-FBEM-NT was evaluated in nude mice bearing HT-29 tumors. Thirty minutes after the intravenous injection of 7.4 MBq of ^{18}F-DEG-VS-NT or ^{18}F-FBEM-NT, the mice were sacrificed. Urine was collected and diluted with 1 mL PBS.

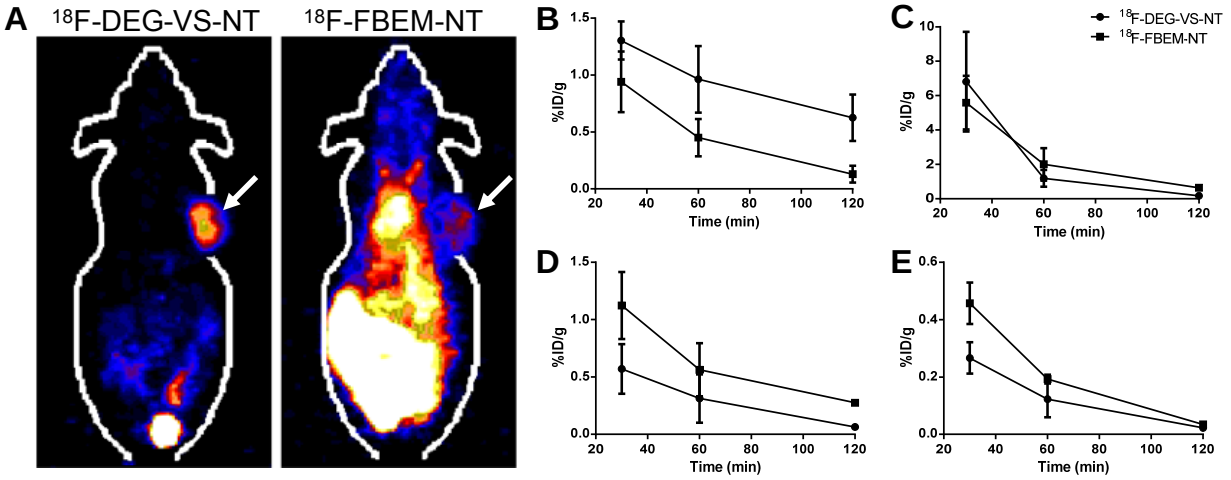
Blood was centrifuged for 5 min at 14,000 rpm. Liver, kidneys, and tumor were harvested and homogenized using a homogenizer, suspended in 1 mL of PBS buffer, and then centrifuged for 5 min at 14,000 rpm. For each sample, after the removal of the supernatant, 50% TFA in 100 μL PBS was added to the solution, followed by mixing and centrifugation for 5 min. The upper solution was then taken and injected for HPLC analysis (HPLC Method 3). The eluent was collected with a fraction collector (1.0 min/fraction), and the radioactivity of each fraction was measured with a gamma counter. For ^{18}F-DEG-VS-*c*(RGDyC) and ^{18}F-FBEM-*c*(RGDyC), the urine samples were collected and analyzed by HPLC.



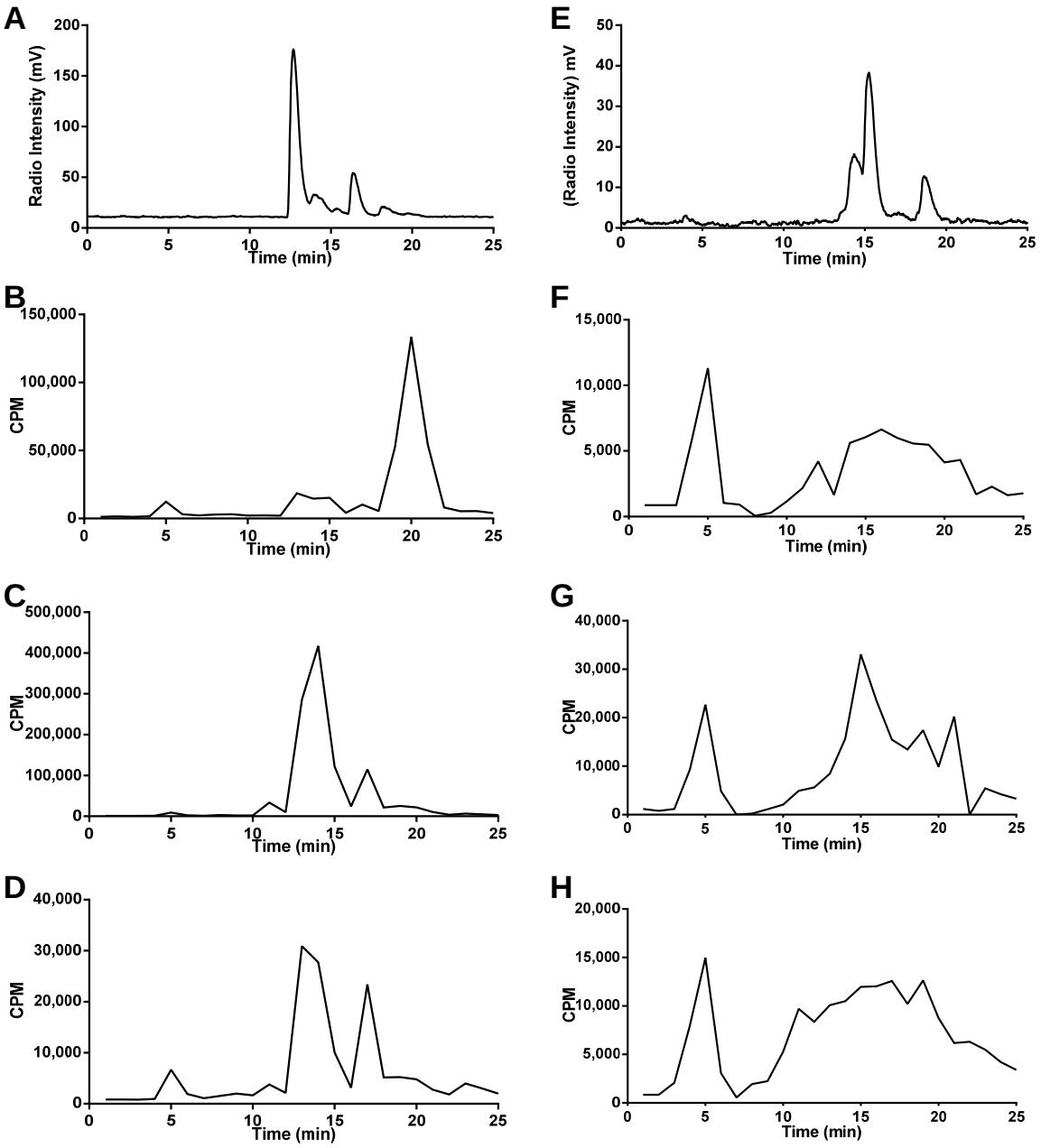
Supplemental Figure 1. Radio HPLC trace of ^{18}F-DEG-VS-NT after 5 h incubation in 1×PBS at 37 °C.



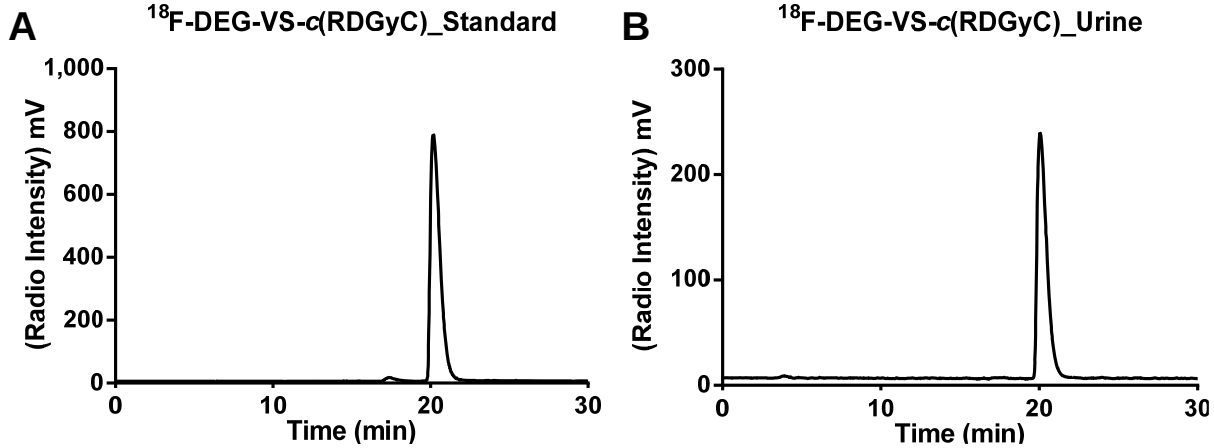
Supplemental Figure 2. (A) Radiosynthesis scheme of ^{18}F-FBEM; (B) Chemical structure of ^{18}F-FBEM-*c*(RGDyC) and ^{18}F-FBEM-NT.



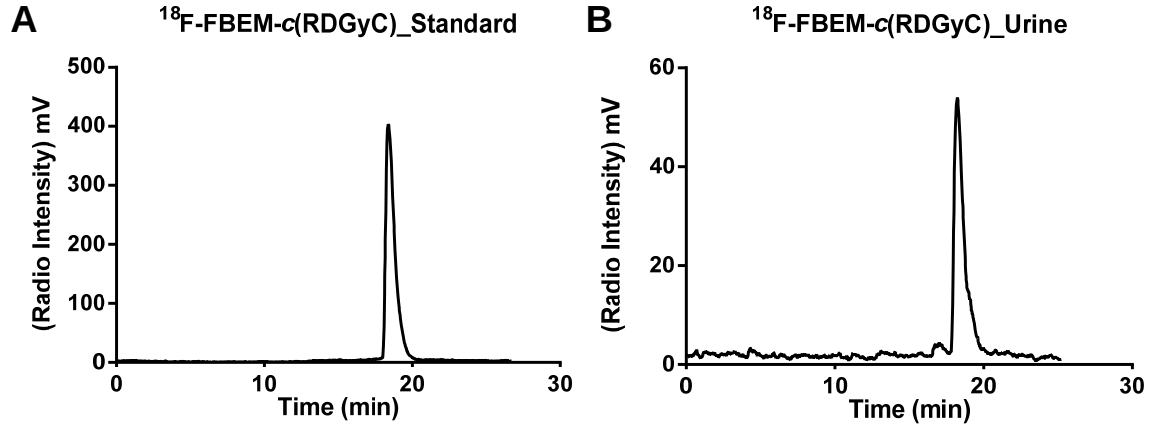
Supplemental Figure 3. (A) 2-D projection microPET images of mice bearing HT-29 tumor at 2 h after injection of ^{18}F-DEG-VS-NT and ^{18}FBEM-NT (arrows indicate HT29 tumors); Time activity curves of tumor (B), kidney (C), liver (D), and muscle (E) after injection of ^{18}F-DEG-VS-NT and ^{18}FBEM-NT.



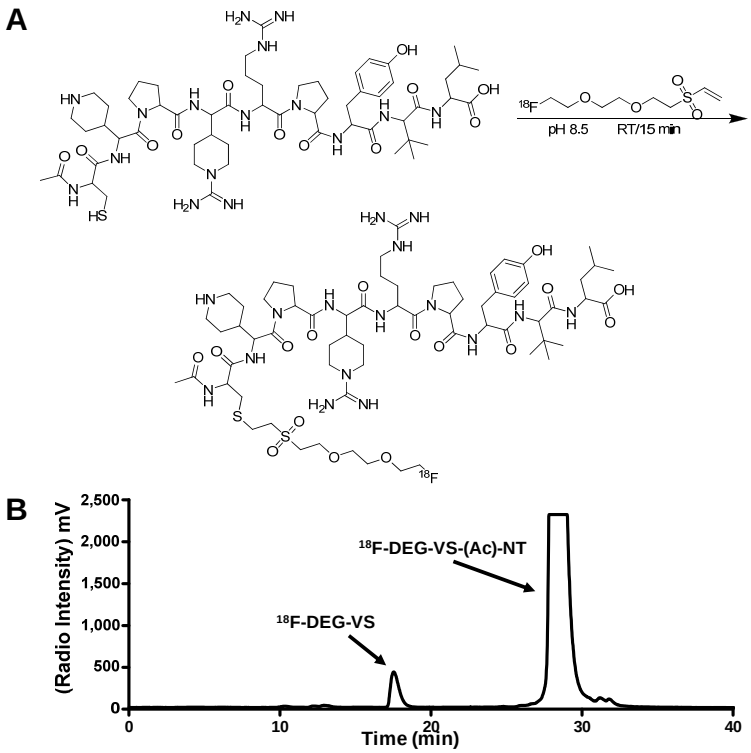
Supplemental Figure 4. Metabolic stability of ^{18}F-DEG-VS-NT (1 h p.i.) in urine (A), tumor (B), kidney (C), and liver (D); and metabolic stability of ^{18}F-FBEM-NT (1 h p.i.) in urine (E), tumor (F), kidney (G), and liver (H). The retention time for ^{18}F-DEG-VS-NT and ^{18}F-FBEM-NT was 18.5 min and 19.0 min, respectively.



Supplemental Figure 5. The radio HPLC profiles of ^{18}F-DEG-VS-*c*(RGDyC) standard (A) and the metabolic stability in urine (B).



Supplemental Figure 6. The radio HPLC profiles of ^{18}F-FBEM-*c*(RGDyC) standard (A) and the metabolic stability in urine (B).



Supplemental Figure 7. (A) Radiosynthesis scheme of ^{18}F-DEG-VS-(Ac)-NT and (B) the corresponding radio-HPLC trace.