EXPERIMENTAL SECTION

Synthetic Procedures and Characterization Data

General Methods

All chemicals were purchased from commercial sources. Analytical TLC was performed with 0.25 mm silica gel 60F plates with fluorescent indicator (254 nm). Plates were visualized by ultraviolet light. The ^{1}H and ^{13}C NMR spectra were performed on a Bruker 400 MHz magnetic resonance spectrometer. Data for ^{1}H NMR spectra are reported as follows: chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform-*d* (δ 7.26, s) or methanol-*d*4 (δ 3.31); multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), or br (broadened); coupling constants are reported as a J value in Hertz (Hz); the number of protons (n) for a given resonance is indicated nH, and based on the spectral integration values. MALDI-MS spectrometric analyses were performed at the Mass Spectrometry Facility of Stanford University.

*Synthesis of the compound **4***

To a solution of *N*-Boc-propargylglycine (384 mg, 1.8 mmol) in THF (3 mL) was added 4-methylmorpholine (330 mL, 3.0 mmol) and isobutyl chloroformate (195 mL, 1.5 mmol) at 0 ºC. The resulting mixture was stirred for 2 h at 0 ºC, then 6-amino-2-cyanobenzothiazole (175 mg, 1.0 mmol) in THF (5 mL) was added at 0 ºC. The resulting solution was stirred for 12 h at room temperature. The reaction was quenched by aqueous 1 M HCl (3 mL). The resulting solution was partitioned with EtOAc (40 mL) and H_2O (40 mL) and the organic phase was washed with water and aqueous $NaHCO_3$ (40 mL). The combined organic phase was dried with $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified with silica gel chromatography (Hexane: EtOAc = 1:1) to give the product **4** (296 mg, 85%).

^{1}H-NMR ($CDCl_3$, 400 MHz) d 9.45(s, 1H), 8.44 (s, 1H), 7.85 (d, 1H, *J* = 8.4), 7.27 (d, 1H, *J* = 8.4), 5.82 (d, 1H, *J* = 7.6), 4.57 (m, 1H), 2.75 (m, 2H), 2.11 (t, 1H, *J* = 2.4), 1.45 (s, 9H); ^{13}C-NMR ($CDCl_3$, 100 MHz) d 170.1, 156.6, 148.5, 138.5, 136.6, 135.3, 125.2, 120.8, 113.2, 111.4, 81.6, 78.8, 72.5, 54.1, 28.6, 22.4; ESI-MS calcd for $C_{18}H_{19}N_4O_3S^+$ ($[M+H]^+$) 371.43, found 371.20.

*Synthesis of the dipeptide **5***

To a solution of the compound **4** (300 mg, 0.81 mmol) in DCM (3 mL) was added anhydrous TFA (3 mL) at room temperature. The resulting solution was stirred for 1 h and then the solvent was evaporated under reduced pressure. The remaining TFA in crude product was removed under vacuum. Dried crude product was dissolved in DMF (5 mL) and *N*-Boc-*S*-Trt-D-cysteine (394 mg, 0.85 mmol), HBTU (323 mg, 0.85 mmol) and DIPEA (442 mL, 2.5 mmol) was added at room temperature. The resulting solution was stirred for 1 h before diluted with EtOAc (30 mL). The resulting organic solution was washed with water (40 mL) and brine (40 mL x 2). The combined organic phase was dried with $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified with silica gel chromatography (Hexane: EtOAc = 1:1) to give the dipeptide **5** (464 mg, 80%).

^{1}H-NMR ($CDCl_3$, 400 MHz) d 9.12 (s, 1H), 8.66 (s, 1H), 8.00 (d, 1H, *J* = 8.8), 7.71 (d, 1H, *J* = 8.8), 7.42 (d, 6H, *J* = 7.2), 7.23 – 7.34 (m, 9H), 6.57 (d, 1H, *J* = 8.4), 5.02 (d, 1H, *J* = 5.2), 4.70 (m, 1H), 3.23 (m, 1H), 3.02-3.08 (m, 1H), 2.93 (dd, 1H, *J* = 13.2, 8.4), 2.62 – 2.69 (m, 2H), 2.02 (t, 1H, *J* = 2.4), 1.40 (s, 9H); ^{13}C-NMR ($CDCl_3$, 100 MHz) d 171.5 168.6, 156.1, 149.0, 144.3, 138.6, 136.8, 135.5, 129.7, 128.5, 127.4, 125.2, 121.6, 113.3, 112.0, 81.4, 78.9, 72.5, 67.8, 54.9, 52.2, 33.3, 28.5, 21.4; ESI-MS calcd for $C_{40}H_{37}N_5NaO_4S_2$ ($[M+Na]^+$) 738.22, found 738.19.

*Synthesis of the compound **7a***

To a solution of the compound **5** (143 mg, 0.2 mmol) in DMF (5 mL) was added the quinoline **6a**[1] (89 mg, 0.2 mmol), TCEP•HCl (115 mg, 0.4 mmol) and DIPEA (418 mL, 2.4 mmol). The resulting solution was stirred for 30 min at room temperature and then the solvent was evaporated under reduced pressure. To remove both *N*-Boc and *S*-Trt group, the crude product was dissolved in the mixture of DCM:TFA:TIPS (1: 1: 0.05) and the resulting solution was stirred at room temperature. After 1 h, the solvent was evaporated under reduced pressure and the remaining TFA was further removed under vacuum. The dried product was re-dissolved in MeOH (5 mL) and 2-(ethyldisulfanyl)pyridine (68 mg, 0.4 mmol) was added. The resulting solution was stirred for 2 h at room temperature. The crude product was purified with preparative HPLC to give the desired product **7a** (88 mg, 51% from **5**).

^{1}H-NMR (CD_3OD, 400 MHz) d 8.30 (d, 1H, *J* = 2.0), 8.12 (d, 1H, *J* = 8.8), 7.86 (d, 1H, *J* = 8.8), 7.78 (d, 1H, *J* = 9.2), 7.61 (d, 1H, *J* = 8.8), 7.51 (dd, 1H, *J* = 9.2, 2.4), 7.39 (dd, 1H, *J* = 9.2, 2.4), 7.15 (d, 1H, *J* = 2.4), 5.30 (t, 1H, *J* = 9.2), 4.77 (t, 1H, *J* = 7.2), 4.32 (dd, 1H, *J* = 8.8, 4.8), 4.12-

4.18 (m, 2H), 3.68-3.78 (m, 2H), 3.64 (m, 1H), 3.48 (m, 1H), 3.35 (dd, 1H, *J* = 14.4, 4.8), 3.09 (dd, 1H, *J* = 14.4, 8.8), 2.76-2.93 (m, 4H), 2.52 (t, 1H, *J* = 2.4), 2.04-2.18 (m, 2H), 1.37 (t, 1H, *J* = 7.6); ^{13}C-NMR (CD_3OD, 100 MHz) d 172.7, 170.4, 168.8, 167.2, 160.9, 160.5, 150.6, 145.2, 138.9, 137.9, 137.5, 131.8, 131.6, 131.4, 126.0, 125.3, 125.0, 121.0, 118.9, 113.0, 106.9, 80.6, 79.9, 73.1, 67.5, 54.7, 53.3, 39.8, 37.9, 35.9, 32.9, 29.3, 22.9, 14.6; MALDI-TOF calcd. for $C_{34}H_{34}N_8NaO_4S_4^+$ ($[M+Na]^+$) 769.934, found 770.139.

*Synthesis of the compound **7b***

To a solution of the compound **5** (72 mg, 0.1 mmol) in DMF (3 mL) was added the quinoline **6b**[1] (42 mg, 0.1 mmol), TCEP•HCl (58 mg, 0.2 mmol) and DIPEA (209 mL, 1.2 mmol). The resulting solution was stirred for 30 min and then the solvent was evaporated under reduced pressure. The crude product was dissolved in the mixture of DCM:TFA:TIPS (1:1:0.05) solution and stirred at room temperature to remove both *N*-Boc and *S*-Trt group. After 1 h, the solvent was evaporated under reduced pressure and the remaining TFA was removed under vacuum. The dried product was re-dissolved in MeOH (3 mL) and 2-(ethyldisulfanyl)pyridine (34 mg, 0.2 mmol) was added. The resulting solution was stirred for 1 h at room temperature. The crude product was purified with preparative HPLC to give the desired product **7b** (33 mg, 40% from **5**).

^{1}H-NMR (CD_3OD, 400 MHz) d 8.87 (dd, 1H, *J* = 5.6, 1.6), 8.75 (d, 1H, *J* = 8.8), 8.23 (d, 1H, *J* = 1.6), 7.99 (d, 1H, *J* = 8.8), 7.82-7.87 (m, 2H), 7.64 (dd, 1H, *J* = 9.2, 2.8), 7.55 (dd, 1H, *J* = 8.8, 2.0), 5.33 (t, 1H, *J* = 9.2), 4.73 (t, 1H, *J* = 7.6), 4.35 (dd, 1H, *J* = 8.4, 4.8), 4.12-4.25 (m, 2H), 3.74 (d, 2H, *J* = 9.2), 3.65 (m, 1H), 3.47 (m, 1H), 3.35 (dd, 1H, *J* = 14.4, 4.8), 3.12 (dd, 1H, *J* = 14.4, 8.0), 2.76-2.88 (m, 4H), 2.54 (t, 1H, *J* = 2.4), 2.06-2.22 (m, 2H), 1.36 (t, 1H, *J* = 7.2); MALDI-TOF calcd for $C_{33}H_{36}N_7O_4S_4^+$ ($[M+H]^+$) 722.943, found 722.770.

Synthesis of Ac-Asp(tBu)-Glu(tBu)-Val-Asp(tBu)-OH (*Ac-DEVD-OH, **8a***)

The protected caspase-3 substrate (Ac-DEVD-OH) was prepared by solid phase peptide synthesis. MALDI-TOF calcd. for $C_{32}H_{54}N_4NaO_{12}$ ($[M+Na]^+$) 709.364, found 709.007.

Synthesis of Ac-Asp(tBu)-Glu(tBu)-Val-Asp(tBu)-OH (*Ac-devd-OH, **8b***)

The peptide **8b** (Ac-devd-OH), control peptide probe, was prepared by using D-amino acids. MALDI-TOF calcd. for $C_{32}H_{54}N_4NaO_{12}$ ($[M+Na]^+$) 709.364, found 709.213.

*Synthesis of the activatable probe **3a***

To a solution of **7a** (15 mg, 17.4 mmol) in THF (2 mL) was added to **8a** (18 mg, 26.1 mmol), HBTU (10 mg, 26.4 mmol) and DIPEA (16 mL, 87 mmol). The resulting solution was stirred for 1 h at room temperature and then diluted with EtOAc (30 mL). The resulting organic solution was washed with brine (30 mL x 2). The combined organic phase was dried with $MgSO_4$, filtered and evaporated under reduced pressure. To remove the *t*-Bu groups, the resulting product was dissolved in DCM:TFA:TIPS (1.0:1.0:0.05). After 1 h, the solvent was evaporated under reduced pressure and the crude product was purified with preparative HPLC to give the probe **3a** (15 mg, 12.0 mmol, 69% from **7a**). MALDI-TOF calcd. for $C_{54}H_{63}N_{12}O_{15}S_4^+$ ($[M+H]^+$)1248.409, found 1248.735.

*Synthesis of the control probe **3b***

To a solution of **7b** (13 mg, 15.6 mmol) in THF (1.5 mL) was added to **8b** (22 mg, 31.2 mmol), HBTU (12 mg, 31.2 mmol) and DIPEA (13.5 mL, 78 mmol). The resulting solution was stirred for 1 h at room temperature. After the reaction was completed, the solvent was evaporated under reduced pressure. The crude product was purified with preparative HPLC to give 11 mg (7.9 mmol) of protected product. MALDI-TOF calcd for $C_{65}H_{88}N_{11}O_{15}S_4^+$ ($[M+H]^+$) 1391.718, found 1392.723. The purified product (11 mg, 7.9 mmol) was dissolved in DMF (1 mL) and then TCEP·HCl (4.5 mg, 16 mmol), DIPEA (20 mL, 0.1 mmol) and methyl iodide (1.9 mL, 30 mmol) was added at room temperature. The resulting solution was stirred for 40 min and then the solvent was evaporated under reduced pressure. To remove t-Bu groups, the resulting product was dissolved in DCM:TFA:TIPS (1.0:1.0:0.05) solution. After 1 h, the solvent was evaporated under reduced pressure and the crude product was purified with preparative HPLC to give the control probe **3b** (7 mg, 6.0 mmol, 38% from **7b**). MALDI-TOF calcd. for $C_{52}H_{62}N_{11}O_{15}S_3^+$ ($[M+H]^+$) 1176.358, found 1176.431.

*Synthesis of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**9**)*

Compound **9** was synthesized according to the reported procedure.[2]

^{1}H-NMR ($CDCl_3$, 400 MHz) d 7.80 (d, 2H, J = 8.4), 7.34 (d, 2H, J = 8.4), 4.16 (t, 2H, J = 4.8), 3.55-3.71 (m, 12H), 3.38 (t, 2H, J = 5.2), 2.45 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) d 145.0,

133.2, 130.1, 128.2, 71.0, 70.9, 70.8, 70.3, 69.5, 68.9, 50.9, 21.9; ESI-MS calcd. for $C_{15}H_{24}N_3O_6S^+$ ($[M+H]^+$) 374.14, found 374.18.

*Synthesis of [^{19}F]-**2***

Two units of caspase-3 enzyme was added to 20 μmol of [^{19}F]-**1** in 200 μL of caspase-3 reaction buffer (50 mM HEPES, 10 mM TECP, 100 mM NaCl, 10% glycerol, pH = 7.5). After 2 h at 30 °C, the cyclization product [^{19}F]-**2** was purified with semi-prep HPLC. [^{19}F]-**2** was characterized by mass spectrum. MALDI-TOF calcd. for $C_{40}H_{44}FN_{10}O_7S_3$ ($[M+H]^+$) 892.034, found 892.874.

Radiosynthesis

General

All chemicals unless otherwise stated were commercially available and used without further purification. Purification of **10** was carried on a high-performance liquid chromatography (HPLC) equipped with Dionex 680 pump (Dionex Corporation, USA) and KNAUER UV detector K-2001 (KNAUER, Germany) using a Phenomenex Gemini C18 column (250 × 10 mm, 5 μm) and gradient conditions (**method A**): A: H_2O + 0.1% TFA, B: CH_3CN + 0.1% TFA; 0-2 min 5% B, 2-30 min 5-65% B, 30-40 min 60-95% B; 5.0 mL/min. **1** and **1-D** was purified on a Dionex Ultimate 3000 chromatography system with a UVD 340U absorbance detector (Dionex Corporation, USA) and model 105S single-channel radiation detector (Carroll & Ramsey Associates, USA) using a Phenomenex Gemini C18 column (250 × 10 mm, 5 μm) and gradient conditions: for **1** (**method B**), A: H_2O + 0.1% TFA, B: CH_3CN + 0.1% TFA; 0-2 min 10% B, 2-40 min 10-60% B, 40-50 min 95% B, 50-60 min 10% B; 5.0 mL/min; for **1-D** (**method C**), A: H_2O + 0.1% TFA, B: CH_3CN + 0.1% TFA; 0-2 min 10% B, 2-40 min 10-40% B, 40-50 min 95% B, 50-60 min 10% B; 5.0 mL/min. Analytical HPLC were performed on an Agilent 1200 Series HPLC system (Agilent Technology, USA) with ChemStation software (version B.04.02) equipped with a quaternary pump, UV diode array detector and model 105S single-channel radiation detector using a Phenomenex Gemini C18 column (250 × 4.6 mm, 5 μm) and gradient conditions: for **1** (**method D**), A: H_2O + 0.1% TFA, B: CH_3CN + 0.1% TFA; 0-2 min 20% B, 2-25 min 20-80% B, 25-28 min 95% B, 28-32 min 20% B; 1.0 mL/min; for **1-D** (**method E**), A: H_2O + 0.1% TFA, B: CH_3CN + 0.1% TFA; 0-2 min 10% B, 2-10 min 10-15% B, 10-25 min 15-25% B, 25-28 min 95% B, 28-32 min 10% B; 1.0 mL/min with UV chromatograms recorded at

214 and 254 nm. The identity of the ^{18}F-labeled products was confirmed by comparison with the analytical HPLC retention time of their non-radioactive reference molecules or by co-injection before administration to animals.

*Synthesis of [^{18}F]azide (**10**)*

10 was fully-automated synthesized in a Tracerlab FX-FN module (GE Healthcare, USA).

Briefly, no-carrier added [^{18}F]fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction by irradiation of enriched [^{18}O]H_2O in a PETtrace cyclotron (GE Healthcare, USA). [^{18}F]Fluoride was trapped on an anion-exchange resin cartridge (Macherey-Nagel Chromafix 30-PS-HCO_3 pre-conditioned with 1 mL of EtOH, 1 mL of H_2O and then blown dry). The cartridge was eluted with a solution of Kryptofix 222 (4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, or K2.2.2) (15 mg) and potassium carbonate (3 mg) in H_2O (0.1 mL) and CH_3CN (0.9 mL). Following azeotropic drying, the compound **9** (3.0 mg in 1.0 mL of dry DMSO) was added to the K[^{18}F]F/K2.2.2 complex, and the mixture was heated for 20 min at 110 °C to yield **10**. After cooling to room temperature, the reaction mixture was loaded on a semi-prep HPLC (**method A**). The fraction corresponding to the peak of the desired product (retention time ~21 min) was collected in a round bottom flask containing sterile water (20 mL), and then transferred to an adjacent customized module for solid phase extraction (SPE) using a C-18 Sep-Pak. **10** trapped on C-18 cartridge was eluted with diethyl ether (2 mL) through a Na_2SO_4 cartridge into a 5 mL V-vial with stirrer bar in the customized module. The diethyl ether was removed under helium stream at ambient temperature and the dried labeling agent was reconstituted with THF (50 μL) for further click chemistry.

*Synthesis of the activatable tracer **1** ([^{18}F]C-SNAT)*

Click chemistry assisted by $Cu(CH_3CN)_4PF_6$ and BPDS:

To a solution of **10** in THF (50 mL) was added the precursor **3a** (0.6 mg, 0.5 mmol, in 25 mL of DMF), $Cu(CH_3CN)_4PF_6$ (50 mL of 200 mM in MeCN) and BDPS (bathophenanthrolinedisulfonic acid disodium salt trihydrate, 100 mL, 50 mM in PB buffer, pH = 7), and the reaction mixture was kept at 60 ºC for 30 min. Crude product was diluted with 2 mL water and injected on semi-prep for purification (**method B**). The fraction corresponding to the peak of the desired product (retention time at ~26 min) was collected and diluted with 20 mL

of water; this solution was loaded on the C-18 light cartridge and eluted out first with 1 mL saline/water (50/50) and then another 4 mL of saline. Both eluted solutions were combined as final product **1**.

Click chemistry assisted by $CuSO_4$ and sodium ascorbate**:**

To a solution of **10** in THF (50 mL) was added **3a** (1.2 mg, 1 mmol) in 1 M HEPES solution (100 mL), $CuSO_4$ (300 mM, 10 μL) and sodium ascorbate (300 mM, 10 μL), and reaction mixture was kept at 40 ºC for 20 min. Crude product was diluted with 2 mL of water and injected on a semi-prep HPLC for purification (**method B**). Final product **1** was formulated in saline with 10% ethanol by SPE (Figure S2A & B).

*Synthesis of the control tracer **1-D***

Click chemistry assisted by $CuSO_4$ and sodium ascorbate**:**

To a THF solution of **10** (50 mL) were added 1 M HEPES/DMSO (1:1) solution (50 mL) of **3b** (0.5 mg, 0.4 mmol), $CuSO_4$ (300 mM, 14 μL) and sodium ascorbate (300 mM, 14 μL), and the reaction mixture was kept at 40 ^{o}C for 20 min. An acetonitrile solution of **11** (250 mM, 200 mL) was added into reaction mixture and kept at 40 °C. The reaction was monitored by analytical HPLC (Figure S1), and the crude product was diluted with 2 mL water and injected on a semi-prep HPLC for purification (**method D**). Product fraction was eluted out at ~42 min and the final product **1-D** was formulated in saline with 10% ethanol by SPE (Figure S2C & D).

*Characterization of [^{18}F]-**2***

Synthesized [^{19}F]-**2** and radiolabeled [^{18}F]-**2** showed the same retention time on analytical HPLC and thus the cyclization product [^{18}F]-**2** from [^{18}F]C-SNAT **1** was characterized based on its retention time on HPLC.

Cell uptake

HeLa cells (cultured in DMEM with 10% FBS and 1% Ampicillin) were grown in a 6 well plate culture dish for 24 h. The wells were then divided into 3 groups. Cells in the group 1 were cultured normally for 24 h, then the medium was changed and the cells were cultured for another 24 h. Group 2 and 3 cells were cultured in the presence of 2 μM doxorubicin for 24 h, then the medium was changed and the cells were cultured for another 24 h: group 2 in normal culture medium without doxorubicin and group 3 in normal culture medium without doxorubicin but

together with 50 μM Z-VAD-FMK a caspase-3 inhibitor. After 48 h all 3 groups were added 10 μCi (0.37 MBq) of **1** and incubated for additional 4 h. Then the medium was removed, the cells washed 3 times with phosphate buffered saline, and 1mL of Tryp-LE^{TM} Express was added and the cells were left for 1-2 min to allow to detach. The cell/Tryp-LE solution was counted in a gamma counter and the radioactivity was normalized to the protein concentration (determined by comparison with BSA standards in a absorption assay from Pierce) in each vial.

Cell uptake HPLC analysis

In a second experiment, following the same procedure, the cell/Tryp-LE solution were placed in a veil and centrifuged at 1200g for 2 min, the supernatant were removed and the pellets were homogenized with a sonicator (Bronson Sonifier 150) in 1 mL of MeOH. The supernatant was collected and analyzed on HPLC (**method D** for **1**; **method E** for **1-D**). Compound **1** and cyclization compound **2** were run on HPLC as well for comparison. The pellets and part of the MeOH supernatants were saved and counted for radioactive content.

Caspase-3 level in apoptotic cells

In a similar experiment to the above, HeLa cells were incubated with different concentrations of doxorubicin (0, 1, 2 and 5 μM) and the cell/Tryp-LE solution were centrifuge at 11,750 g for 5 min, the supernatant was removed and 1 mL/g Riba buffer (Sigma) was added. The cells were left for 15 min on ice and homogenized with a sonicator (Bronson Sonifier 150) and the suspension was analyzed for Caspase-3 activity with a Caspase-GLO® kit from Promega (Figure S4).

Stability in mouse serum

To the mouse serum (500 μL) was added 100 μL (~100 μCi/3.7 MBq) of radioactive sample and the mixture was incubated at 37 ºC. At each time point 100 μL of sample was taken and after mixing with 100 μL of acetonitrile was centrifuged at 9,400g for 5 min. The resulting supernatants were collected, and an aliquot of each supernatant (100 μL) was diluted with 100 μL of water and analyzed via the same HPLC method used for quality control (fitted with a highly sensitive positron detector for radioactivity). The percent ratio of intact probe to the total radioactivity on the HPLC was calculated as % = [(peak area for intact compound)/(total peak area)] × 100 (Figure S3).

Animal model

All experimental procedures involving animals were approved by the Stanford Administrative Panel on Laboratory Animal Care (APLAC). Mice were injected s.c on the right shoulder with an estimated 1-2 million HeLa cells. The HeLa cells were allowed to establish a tumor xenograft at the size of 1 cm in diameter. A subset of the mice was treated with intratumoral injections of 0.2 mg (in 20 μL) doxorubicin. Four days later, the animals were killed, the tumors removed and quickly frozen using dry ice. The following day, tumors were cut into small pieces weighted and 1 mL/g Riba buffer was added, they were kept on ice for 30 minutes and then homogenized with sonicator (Bronson Sonifier 150). The lysed tumor solutions were centrifuged at 11,750 g for 15 min at 4 ºC. The supernatant was analyzed for caspase-3 activity using the Caspase-GLO® kit (Promega and for protein concentration using the standard BSA absorption assay from Pierce).

PET imaging

One group of animals was scanned at baseline (before treatment) and 4 days post treatment with 0.2 mg doxorubicin injected into the tumor. One the day of scanning the animals were moved from their housing area to the PET scanner around 1 hour prior to the experiment. They were constrained shortly in a tail veiner and injected i.v. with 440 ± 97 μCi (16 ± 3 MBq) [^{18}F]C-SNAT formulation. They were anesthetized with 2% isoflurane shortly before placement in an R4 microPET scanner (Siemens Medical Solutions, USA), the anesthesia were continued with 2% isoflurane doing 5 min of acquisition. Scans using the control tracer **1-D** (173 ± 73 μCi / 9 ± 4 Mbq in 200μL formulation) was carried out in a similar manner using two groups (one baseline and one treated) instead of the same animals at baseline and after treatment. The images were reconstructed using a standard 2D reconstruction without attenuation correction.

The reconstructed images were analyzed by making volumes of interest (VOI), one on the tumor and one located on the left shoulder containing the muscle/bone. The percent of injected dose per gram tissue (%ID/g) was calculated in each VOI and the ratio between tumor and muscle uptake was calculated based on this uptake (uptake in tumor/uptake in muscle region). The effect of treatment was calculated as the difference in uptake of [^{18}F]C-SNAT at baseline and after treatment.

Two MIP (maximum projection video's) were made from static PET images (one baseline and one treated) taken two hours after the tracer injection, and fused with a 3D CT image of a nomal nude mouse (Supportive Information video).

Biodistribution

Mice were administered [^{18}F]C-SNAT (100-200 μCi/3.7-7.4 MBq in 100-200 μL of 0.9% saline solution) via tail vein injection and then euthanized by bilateral thoracotomy under anesthesia at 2 h after injection of tracer (n = 3). All tissues (tumor, heart, whole brain, gallbladder, kidney, liver, lung, muscle, bone, spleen, whole blood) were removed quickly following euthanasia and placed in pre-weighed test tubes. Standard solutions of the tracer were prepared by diluting approximately 100 μCi (3.7 MBq) tracer in 100 mL of water, of which 1 mL of aliquots were used for reference counting.

The test tubes containing tissue samples were weighed and the radioactivity was measured by an automated gamma counter (Cobra Model 5002, Packard, USA) along with the standard solutions. The ratios of the radioactivity in organs to muscle were shown in table S1.

Statistical analysis

Comparisons were done using a one-sided unpaired t-test, except that a one-sided paired t-test was used to compare treated to baseline of [^{18}F]C-SNAT.

SUPPLEMENTARY FIGURES AND TABLES

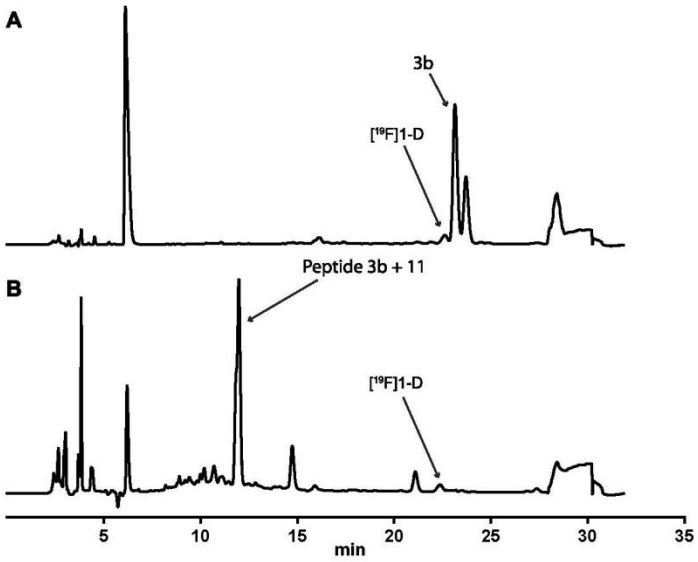

Figure S1 Representative analytical HPLC chromatogram (**method E**) (UV at 254 nm) for monitoring CuAAC in the synthesis of **1-D**. A) Aliquot of click chemistry reaction of **3b** and **10** after 30 min; B) Aliquot of reaction mixture 30 min after adding **11 (**3-azidopropan-1-amine hydro-chloride**)**.

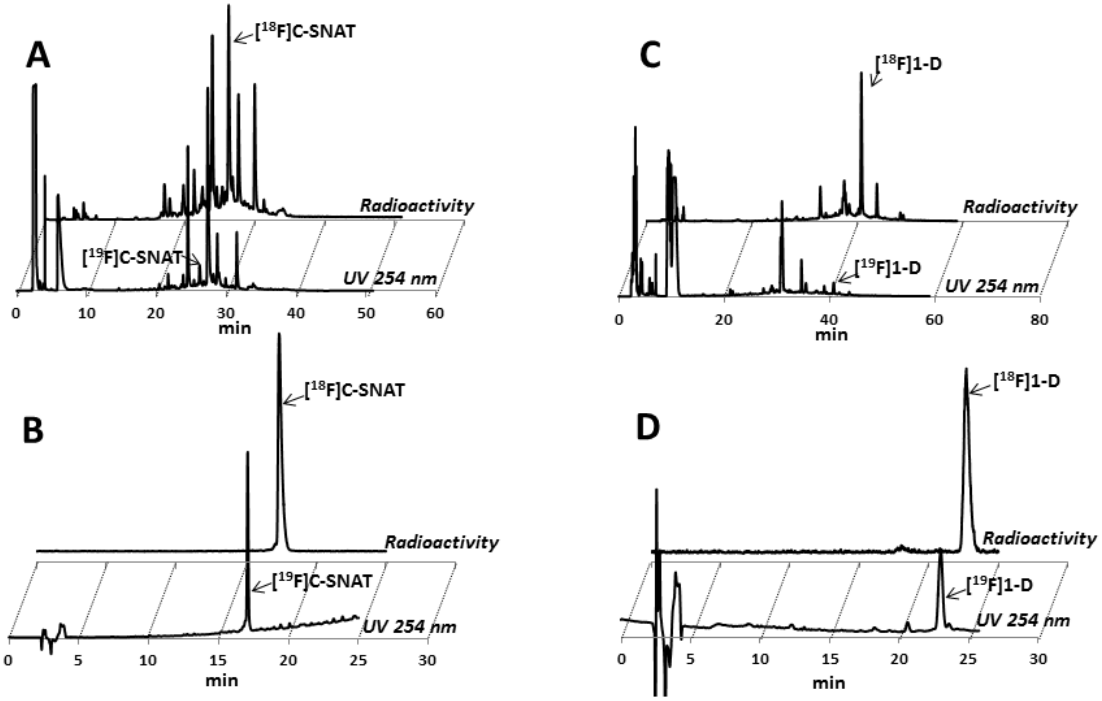

Figure S2 A) Semi-Prep HPLC chromatogram of crude [^{18}F/^{19}F]**C-SNAT**; B) Analytical HPLC chromatogram of purified [^{18}F/^{19}F]**C-SNAT**; C) Semi-Prep HPLC chromatogram of crude [^{18}F/^{19}F]**1-D**; D) Analytical HPLC chromatogram of purified [^{18}F/^{19}F]**1-D**.

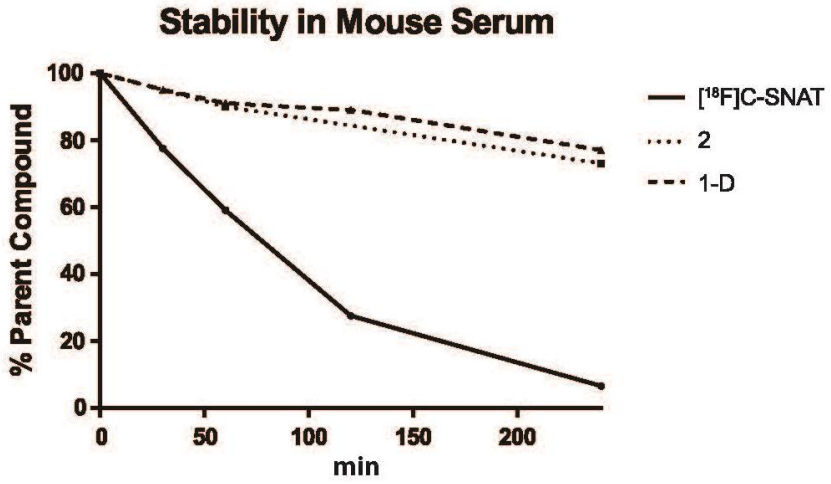

Figure S3 Stability of **[^{18}F]C-SNAT (1)**, **1-D** and **2** in mouse serum.

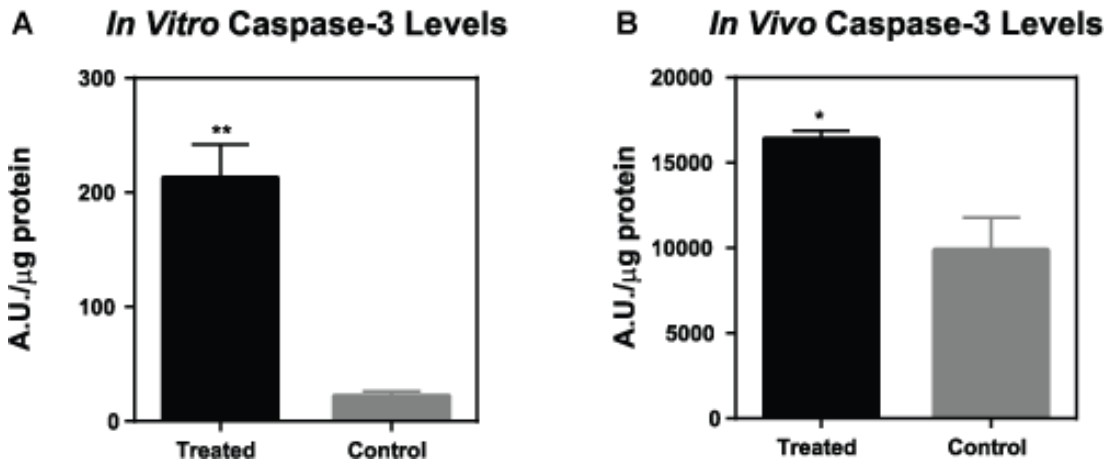

Figure S4 A) Caspase-3 level in cell culture; Levels in treated cells are significantly higher than control (p=0.0014); B) Caspase-3 level in tumor; Levels in treated tumor are significantly higher than control (p=0.0149).

Table S1 Ex vivo biodistribution data of [^{18}F]C-SNAT (**1**) 2 h post injection (ratio of %ID/g over muscle ± SEM).

Region (n)	Ratio over muscle
Muscle (5)	1.0 ± 0.5
Bone (5)	2.4 ± 1.5
Brain (5)	0.5 ± 0.2
Hearth (3)	4.5 ± 2.4
Kidney (5)	124.2 ± 46.6
Liver (5)	22.9 ± 9.7
Lung (3)	4.1 ± 1.8
Plasma (2)	4.0 ± 1.8
Spleen (5)	1.7 ± 0.7
Tumor (2)	5.6 ± 2.3
Whole Blood (5)	5.1 ± 2.4

Table S2 Comparison of apoptosis PET tracers.

Tracer	Species	Probe mechanism	Origin of apoptosis	%ID/g at the target site	Target / background[a]	Treat / control	Reference
[^{18}F]C-SNAT	Mouse	Caspase-3 activated	Tumor treatment	1.3 ± 0.1	7	1.9	This work
[^{18}F]Annexin V	Mouse	PS binding	Tumor treatment	1.6 ± 0.2	2.7	1.8	Hu *et al.* 2012[3]
[^{18}F]ICMT-11	Mouse	Caspase-3 inhibitor	Tumor treatment	0.5	1.2[b]	1.7	Nguyen *et al.* 2009[4]
[^{18}F]WC-IV-3	Rat	Caspase-3 inhibitor	Liver apoptosis	2.75 ± 0.60	N/A	3.5	Chen *et al.* 2012[5]
[^{18}F]ML-10	Mouse	Membrane potential	Cerebral stroke	0.34 ± 0.13	N/A	2.3	Reshef *et al.* 2008[6]
[^{18}F]CP18	Mouse	Caspase-3 activated	Thymus apoptosis	N/A	~10[c]	4.3	Su *et al.* 2013[7]

[a] The maximum values reported at the apoptotic tissue; [b] Tumor/blood; [c] Thymus/muscle. PS: phosphatidylserine.

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