Supplemental Material

Reagents. Common reagents were purchased from Sigma-Aldrich (St. Louis, MO) or Acros (Geel, Belgium) and used as received unless otherwise specified. Annexin A5 was obtained from Theseus Imaging Corp. (Cambridge, MA). *p*-Isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (DTPA-Bz-NCS) was purchased from Macrocyclics (Dallas, TX). Indium-111 chloride ($^{111}InCl_3$) was obtained from Perkin-Elmer (Waltham, MA).

CCPM Nanoparticles. CCPM nanoparticles were a kind gift of Carestream Health, Inc. (Rochester, NY). Each CCPM nanoparticle contained approximately 21 Cy7-like dye molecules and 180 amino (NH_2) groups. DTPA loading was estimated to be approximately 19 DTPA molecules per nanoparticle. The detailed procedure for synthesis of CCPM nanoparticles was described in a previous report (*29*).

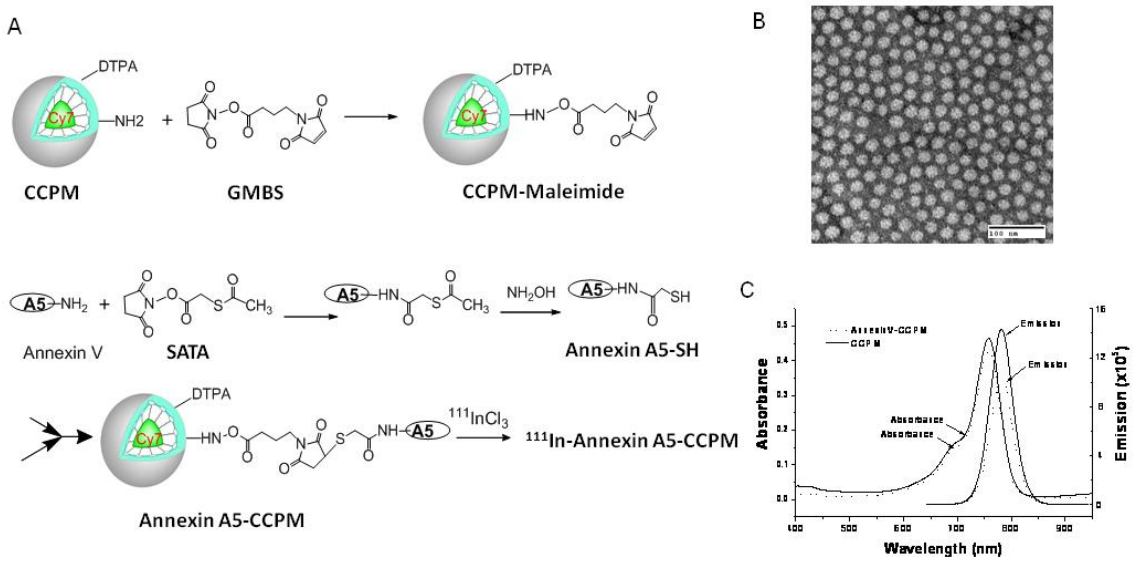
Annexin A5-Functionalized CCPM. The reaction scheme for the conjugation of annexin A5 to the surface of CCPM is shown in **Suppl. Fig. 1A**. To introduce maleimide group to CCPM, an aliquot of *N*-[γ-maleimidobutyryloxy]-succinimide ester (GMBS) in dimethylformamide (22.5 μL, 7 μmol/mL, 0.16 μmol) was added into CCPM (250 μL, 2.6 nmol nanoparticles) in 1.2 mL of phosphate-buffered saline (PBS, pH 8). The mixture was stirred for 3 h at 37°C. The product was purified using a PD-10 column to remove unreacted GMBS. To introduce sulfhydryl group to annexin A5, an aliquot of *N*-succinimidyl *S*-acetylthioacetate in dimethyl sulfoxide (26.4 μL, 50 nmol/mL, 1.3 μmol) was added into annexin A5 (2.4 mg, 0.06 μmol) in 4.5 mL of PBS (pH 8). The mixture was stirred for 12 h at 4°C, and then hydroxylamine in water (0.5 mL, 0.5 M) was added to the solution to remove the protecting group. The reaction mixture was stirred for an additional 2 h and was concentrated to 1 mL by ultracentrifugation (MWCO, 10K; Millipore Corp., Bedford, MA). After being passed through a PD-10 column to remove small-molecular-weight contaminants, the resulting sulfhydryl-containing annexin A5 was mixed with 2 mL of

PBS solution of CCPM-maleimide (0.16 μmol equivalent maleimide) with a molar ratio of annexin A5 to maleimide groups (CCPM) of 1:2. The solution was stirred for 12 h at 4°C and then purified using a fast protein liquid chromatography system (Amersham Pharmacia Biotech, Sweden) equipped with a G200 column and an ultraviolet light detector (280 nm). The column was eluted with PBS to remove unreacted annexin A5. The unreacted annexin A5 was quantified using a protein assay kit (Bio-Rad, Hercules, CA) according to the manufacturer's protocol, and the data were used to calculate the molar ratio of annexin A5 bound to CCPM in annexin A5-CCPM.

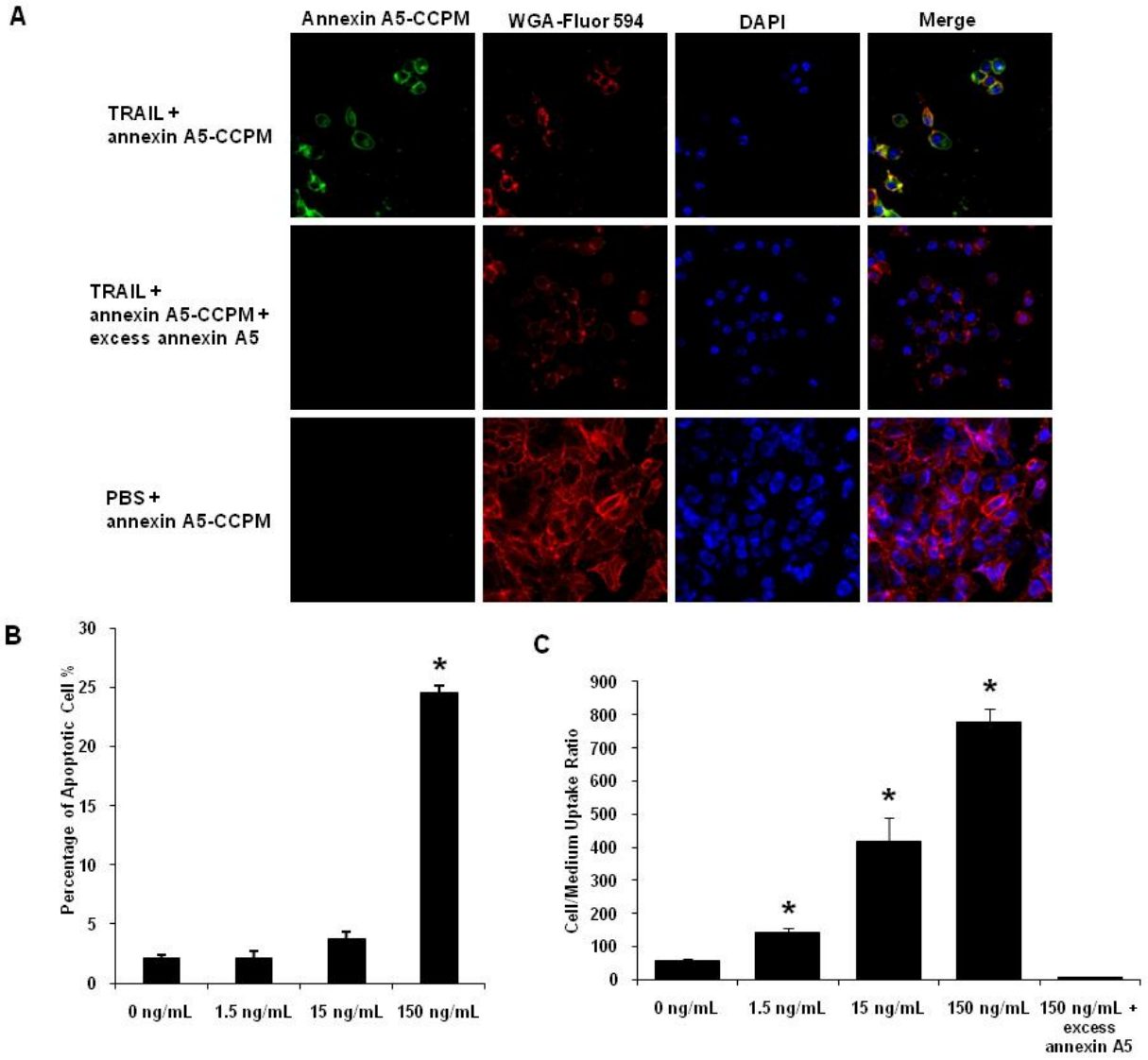
Characterization of Annexin A5-CCPM. For transmission electron microscopic examination, a drop of aqueous sample solution was placed on a 400-mesh copper grid coated with 0.5% poly(vinyl formal) aqueous solution (w/w). Negative staining was performed using a droplet of 1% uranyl acetate solution. The sample was air-dried and examined with a JEM 1010 transmission electron microscope (JEOL USA, Inc., Peabody, MA) at an accelerating voltage of 80 kV. Digital images were obtained using the AMT Imaging System (Advanced Microscopy Techniques Corp., Danvers, MA). Fluorescence spectra were recorded using a Fluorolog fluorometer (Horiba, Edison, NJ).

Radiolabeling. Aliquots of annexin A5-CCPM in 0.1 M sodium acetate solution (pH 5.2) were mixed with an aqueous solution of $^{111}InCl_3$ at room temperature for 30 min. Radiolabeled nanoparticles were analyzed using an instant thin-layer chromatography system. The instant thin-layer chromatography strips were developed with PBS (pH 7.4) containing 4 mM EDTA and quantified using a Bioscan IAR-2000 TLC Imaging Scanner (Washington, DC). Free $^{111}In^{3+}$ moved to the solvent front (Rf = 0.9), and the nanoparticles remained at the original spot (Rf = 0.0).

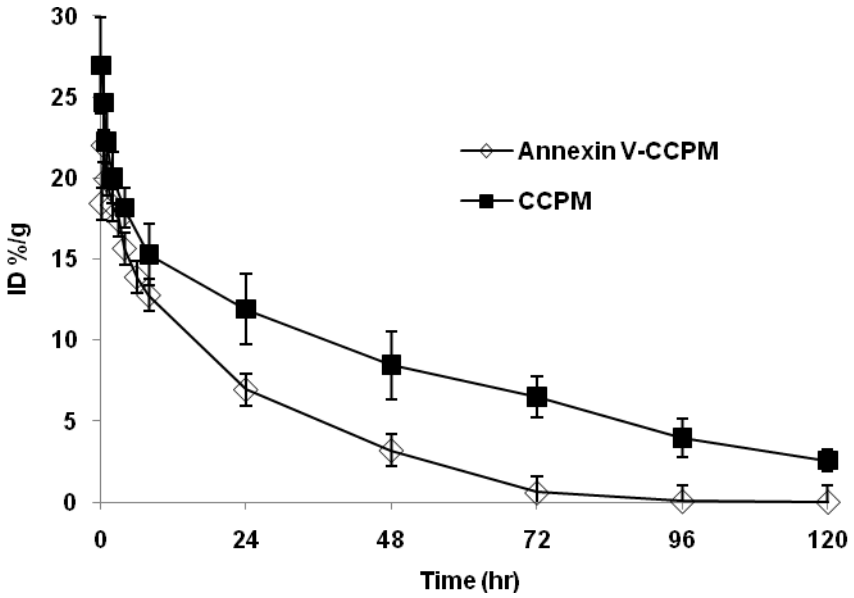
Supporting Data



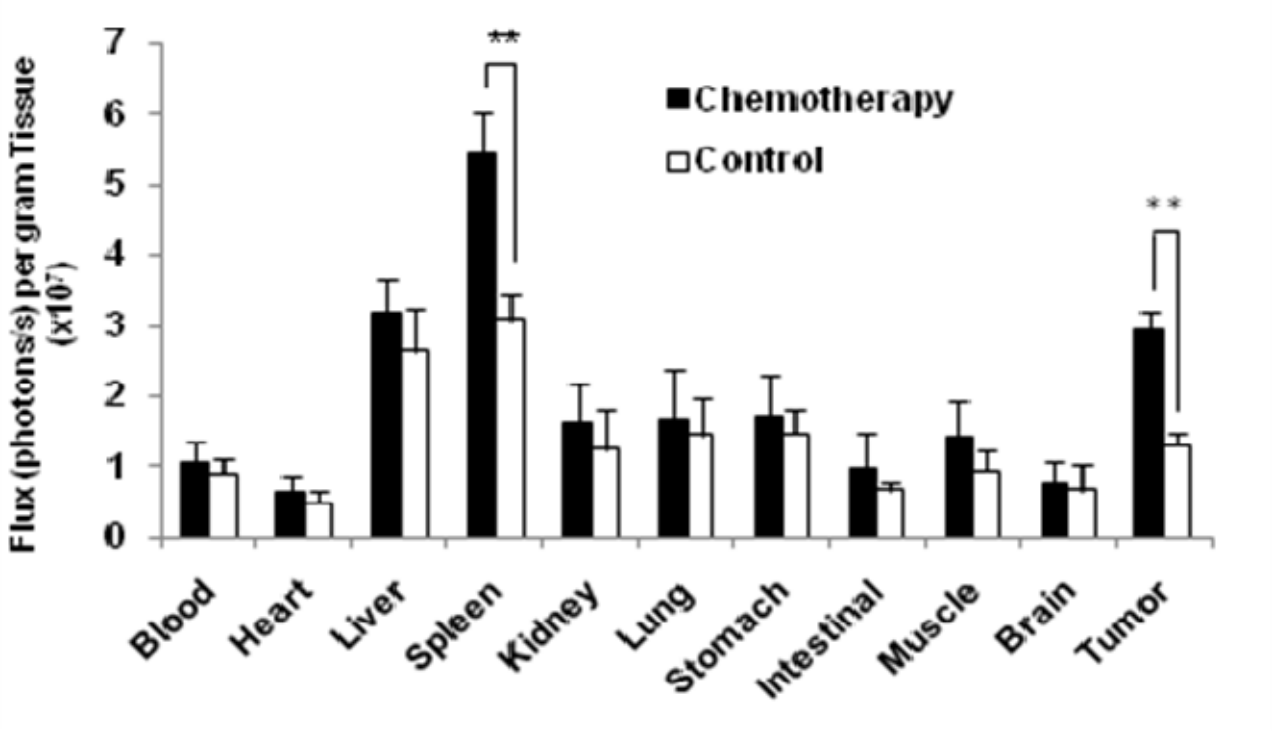
Supplemental Figure 1. Synthesis and characterization of ^{111}In-labeled annexin A5-CCPM nanoparticles. (A) Reaction scheme for the conjugation of annexin A5 to CCPM and radiolabeling of the resulting annexin A5-CCPM. (B) Transmission electron micrograph of annexin A5-CCPM nanoparticles. (C) Absorbance and emission spectra of CCPM before and after introduction of annexin A5.



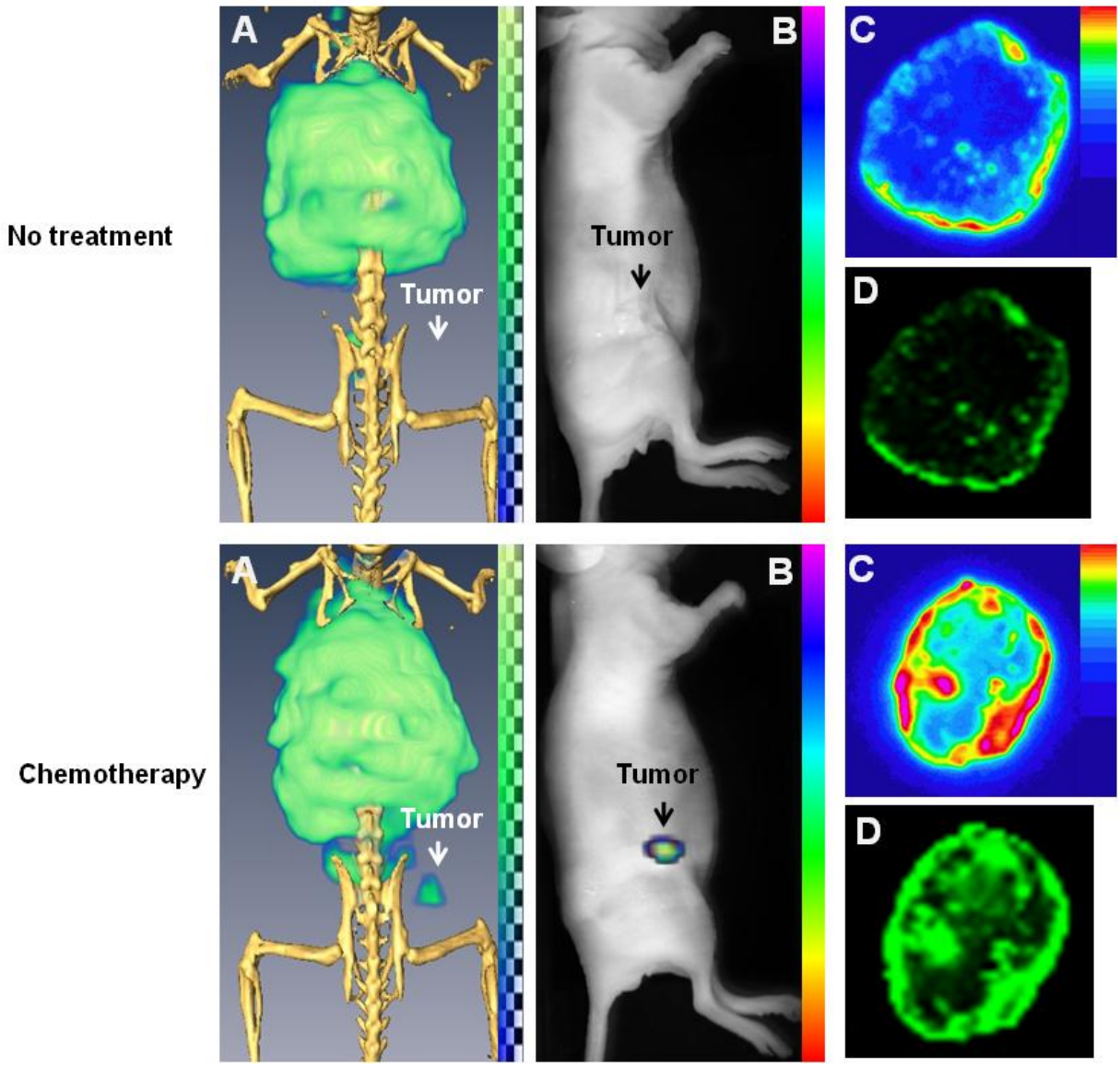
Supplemental Figure 2. Fluorescence microphotographs and Uptake of DLD-1 human colon cancer cells after exposure to ^{111}In-labeled annexin A5-CCPM. In (A), DLD-1 cells were treated with TRAIL (150 ng/mL) followed by ^{111}In-labeled annexin A5-CCPM 2 h later (upper); TRAIL followed by ^{111}In-labeled annexin A5-CCPM plus annexin A5 (100-fold excess) 2 h later (middle); or PBS followed by ^{111}In-labeled annexin A5-CCPM 2 h later (lower). Signal from Cy7-loaded CCPM is pseudocolored green. Cell membrane was stained with Alexa Fluor 594-labeled wheat germ agglutinin (WGA) (red), and cell nuclei were stained with DAPI (blue). (B) DLD-1 cells were incubated with TRAIL for 2 h at the indicated doses, stained with annexin A5-FITC, and assayed for the percentage of apoptotic cells using flow cytometry. (C) DLD-1 cells were incubated with TRAIL for 2 h at the indicated doses and then incubated with ^{111}In-labeled annexin A5-CCPM without or with annexin A5 (100-fold excess) for 15 min. The data are expressed as cpm/μg protein in cell pellet over cpm/μg medium and presented as mean ± standard deviation (n = 5). * Indicates statistically significant change in values with P <0.01.



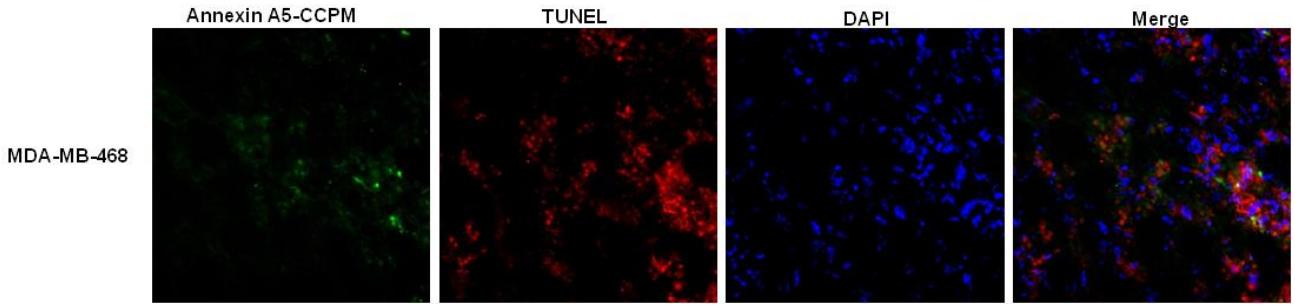
Supplemental Figure 3. Blood activity-time profiles of ^{111}In-labeled annexin A5-CCPM and plain CCPM. The open diamonds represent the mean radioactivity expressed as a percentage of the injected dose per gram of blood from 7 mice. Data for CCPM were taken from reference (*29*).



Supplemental Figure 4. Biodistribution 48 h after the administration of ^{111}In-labeled annexin A5-CCPM in mice bearing EL4 lymphoma. Data obtained using the fluorescence intensity measurement method plotted as photon count per gram of tissue. The mice in the treatment group were injected with ^{111}In-labeled annexin A5-CCPM intravenously 24 h after administration of cyclophosphamide and etoposide. The mice in the control group were injected only with ^{111}In-labeled annexin A5-CCPM. All the data are expressed as mean ± standard deviation (n = 7). ***P* = 0.001.



Supplemental Figure 5. Dual SPECT/CT and near-infrared fluorescence optical imaging of MDA-MB-468 tumor apoptosis with ^{111}In-labeled annexin A5-CCPM. The mice in the chemotherapy group (bottom) received an intravenous injection of ^{111}In-labeled annexin A5-CCPM after treatment with poly(L-glutamic)-paclitaxel conjugate (100 mg eq. paclitaxel/kg by intravenous injection; day 1) and cetuximab (1 mg/mouse by intraperitoneal injection; day 4). The mice in the control group (top) were injected intravenously only with ^{111}In-labeled annexin A5-CCPM. (A) Representative SPECT/CT images. (B) Representative fluorescence molecular tomographic images. (C) Representative autoradiographs of excised tumors. (D) Fluorescence images of the same slides used in autoradiographic studies. All images were acquired 48 h after injection of ^{111}In-labeled annexin A5-CCPM.



Supplemental Figure 6. Fluorescence microscopy of MDA-MB-468 breast tumor from mice treated with chemotherapy. The tumor sections were subjected to TUNEL staining (red). Signal from Cy7 loaded annexin A5-CCPM was pseudocolored green and cell nuclei were stained with DAPI (blue).