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Supporting Information

A histone H1-binding-aptide–based apoptosis-imaging probe for monitoring tumor responses to cancer therapy

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Experimental Section:

Preparation of Cy5.5-conjugated ATP_{HH1} (Cy 5.5-ATP_{HH1}). Seventy-five micrograms of Cy5.5 mono-NHS ester (GE Healthcare Life Sciences, Pittsburgh, PA, USA) and 100 μg of N-terminal acetylated histone H1-binding aptide (Ac-CQRPPRGGGSWTWENGKWTWKGLWYRTL) were dissolved in 45 μl of DMSO containing 2 μl of trimethylamine (TEA; final concentration, 70 mM) and allowed to react in the dark overnight with vigorous stirring. Cy5.5-ATP_{HH1} was purified using semi-preparative reverse-phase high-performance liquid chromatography (HPLC), and the resulting product was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). A Cy 5.5 conjugated scrambled aptide (Cy 5.5-APT_{SCR} having sequence of GGGGSGGSWTWENGK[Cy-5.5]WTWKGGSGGGG) was used as a negative control. The histone H1-binding fluorescence probe, Cy5.5-ApoPep, obtained from BioActs (Incheon, Korea), was also used as a control.

Cell culture. The A549 human alveolar basal epithelial carcinoma cell line, MDA-MB-231 breast adenocarcinoma cell line, and SKOV-3 ovarian adenocarcinoma cell line were obtained from the Korean Cell Line Bank (Seoul, Korea). A549 lung tumor cells were grown in a humidified incubator in a 95% air/5% CO_2 atmosphere at 37°C in Ham's F12K medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 IU/mL penicillin, and 100 IU/ml streptomycin. MDA-MB-231 breast tumor cells and SKOV-3 ovarian tumor cells were cultured in RPMI-1640 medium (Welgene, Daegu, Korea) in a humidified 5% CO_2 atmosphere at 37°C. All cell lines were tested for mycoplasma contamination.

Confocal microscopy. Apoptosis was induced by incubating A549 tumor cells with 150 μM etoposide (Sigma-Aldrich, St. Louis, MO, USA) for 12 h. Necrosis was induced by incubating cells with glucose-depleted medium for 10–15 h. As a negative control, cells were incubated

under the same conditions in normal medium without etoposide. After incubating with 10 mM HEPES buffer (pH 7.4) containing 1% bovine serum albumen (BSA) and 150 mM NaCl at 37°C for 30 min, cells were treated with Cy5.5-ApoPep (10 μM), Cy 5.5-ATP_{SCR} (100 nM) or Cy 5.5-ATP_{HH1} (100 nM) at 4°C for 1 h. Cells were then fixed with 4% paraformaldehyde, stained with 4',6'-diamidino-2-phenylindole (DAPI; Sigma-Aldrich), mounted with fluorescence mounting medium (Dako, Carpinteria, CA, USA), and visualized by confocal laser-scanning microscopy (LSM 710; Carl Zeiss Microimaging, Jena, Germany). For competition assays, cells were pre-treated with 1 mM ATP_{HH1} for 1 h prior to addition of Cy5.5-ATP_{HH1}. For flow cytometry analyses, 1×10^6 cells in suspension were incubated with Cy5.5-ApoPep (10 μM) or Cy 5.5-ATP_{HH1} (100 nM and 1 μM) and then subjected to flow cytometry.

Animals. All animals were obtained from Orient Bio, Inc. (Seongnam, Korea) and were housed under pathogen-free conditions in the animal facility at the Korea Advanced Institute of Science and Technology. All surgeries were performed under isoflurane anesthesia, and all efforts were made to minimize suffering. All animal procedures were reviewed and approved (approval number: KA2013-17) by the Korea Advanced Institute of Science and Technology Institutional Animal Care and Use Committee (KAIST-IACUC) for compliance with ethical procedures and standards of scientific care.

***In vivo* fluorescence imaging of tumor apoptosis.** A tumor xenograft mouse model was prepared by injecting A549 tumor cells (1×10^6), MDA-MB-231 tumor cells (5×10^6), and/or SK-OV-3 tumor cells (5×10^6) subcutaneously into the dorsal flanks (right or both left and right) of 6-wk-old female BALB/c nude mice. When tumor volumes reached at least 200– 300 mm^3, docetaxel (5 and 10 mg/kg) or Herceptin (20 mg/kg) was injected intratumorally or

intravenously via the tail vein. After allowing sufficient time (12 h) for apoptotic regions to develop in target tumors of mice, Cy5.5-ApoPep (25 pmol or 5 nmol) or Cy 5.5-ATP_{HH1} (25 pmol) was intravenously injected via the tail vein. At predetermined times, *in vivo* fluorescence images of mice were acquired under isoflurane anesthesia using a Xenogen IVIS Lumina *in vivo* imaging system (PerkinElmer, Waltham, MA, USA), with a Cy5.5 filter and an exposure time of 5 s. If needed, mice were sacrificed at predetermined times and then major organs (colon, kidney, liver, spleen, lung, and heart) were collected. The fluorescence intensities of organs from mice in each group were analyzed as described for *in vivo* imaging.

Immunohistochemistry of peptide homing to tumors. Cy5.5-ATP_{HH1} (25 pmol) was intravenously injected into the tail vein of mice bearing a subcutaneous tumor 12 h after treatment with docetaxel (10 mg/kg). Six hours later, mice were sacrificed, and tumors were removed, fixed, and cryosectioned. After fixing sections with 4% paraformaldehyde, apoptotic areas of tissue were identified using a modified 2-h TUNEL staining protocol (Chemicon, Billerica, MA, USA), after which tissue sections were incubated for 30 min with FITC-labeled secondary antibodies (Invitrogen, Waltham, MA, USA). Tissue sections were then incubated with the nuclear stain DAPI and slide-mounted using a mounting solution before observation under a confocal microscope.

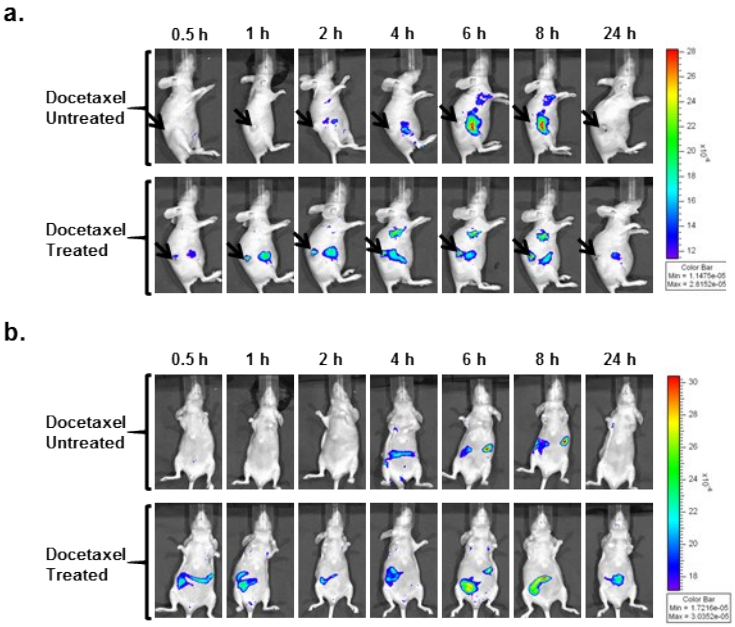

Figure S1. ATP_{HH1} selectively detects apoptotic, anticancer-agent–treated tumors relative to non-apoptotic tumors with a high signal-to-background ratio, even at an extremely low dose. (a, b) A549 tumor xenograft mice were treated with docetaxel (10 mg/kg), administered intratumorally, for 12 h, after which 25 pmol of anti-H1-Cy5.5 was intravenously injected via the tail vein. Lateral (a) and ventral (b) sides of mice were visualized using a Xenogen IVIS Lumina *in vivo* imaging system, with a Cy5.5 filter and an exposure time of 5 s.

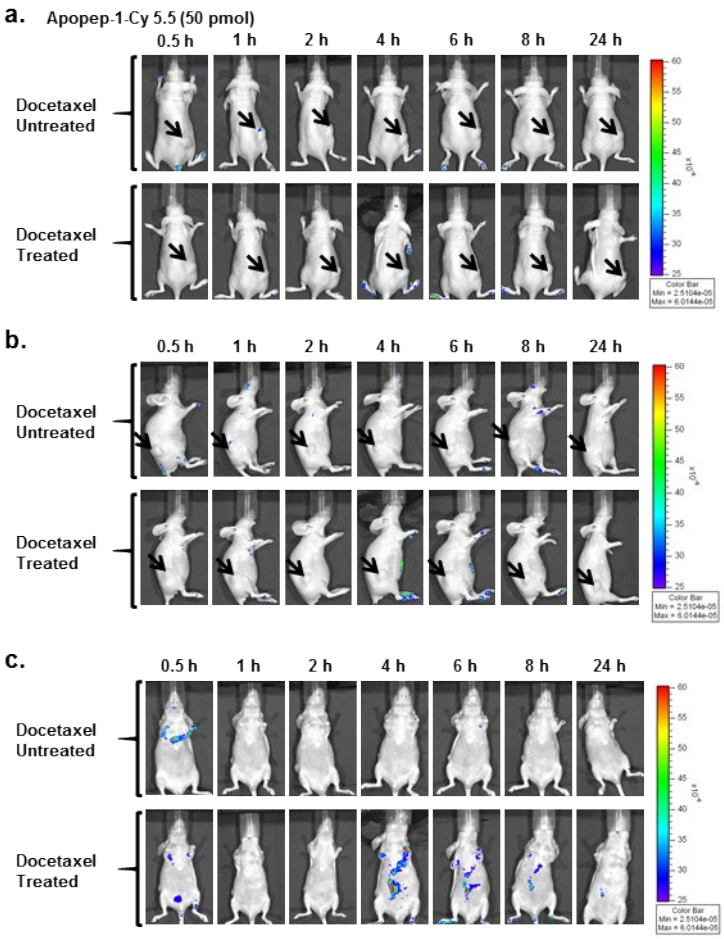

Figure S2. Monitoring anticancer responses of tumors with low-dose (50 pmol) ApoPep. (a – c) A549 tumor xenograft mice were injected with docetaxel (10 mg/kg) by intratumoral injection. After 12 h, mice were injected intravenously with 50 pmol of Cy5.5-ApoPep via the tail vein. Dorsal (a), lateral (b), and ventral (c) sides of mice were visualized using a Xenogen IVIS Lumina *in vivo* imaging system, with a Cy5.5 filter and an exposure time of 5 s.