Supplementary Information

Synthetically Lethal Nanoparticles for Treatment of Endometrial Cancer

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Contents of the Supplementary Information

1 Supplementary materials and methods

1.1 Cell culture and mice

LLC-PK1-WT and LLC-PK1-MDR1 cell lines were generously provided by Dr. John Markowitz from the University of Florida. Both cell lines were maintained in DMEM medium containing 10% FBS, 110 mg/mL sodium pyruvate (Gibco) and 1% Pen/Strep. The blood-brain barrier cell line (hCMEC/D3) was purchased from EMD Millipore and maintained according to the manufacturer's protocol. A20 lymphoma and wild type CT26 colon carcinoma cell lines were purchased from ATCC. A20 lymphoma cells were cultured in RPMI-1640 supplemented with 10% FBS, 1 mM sodium pyruvate (Gibco), 10 mM HEPES (Gibco), 50 µM 2-mercaptoethanol (Sigma), and 50 µg/ml gentamicin sulfate (Mediatech, Inc., VA). CT26 colon cancer cells were cultured in RPMI-1640 supplemented with 10% FBS, 1 mM sodium pyruvate, 10 mM HEPES, and 50 µg/ml gentamicin sulfate. All cell lines were mycoplasma free as determined by MycoAlert mycoplasma detection kit. Female Balb/c mice at the age of 6-8 weeks were purchased from Jackson Laboratories (Bar Harbor, ME).

1.2 Western blot analysis

Western blot analysis was performed as described in section 3.5 of the main manuscript. β-actin (catalogue no. A1978) was purchased from Sigma. Fibroblast Growth Factor Receptor 2 (FGFR2) (catalogue no. 23328) was purchased from Cell Signaling Technology.

1.3 MTS cell proliferation assay

The cell viability analysis was performed as described in section 3.2 of the main manuscript. LLC-PK1-WT and LLC-PK1-MDR1 cells were plated in 96 well plates at a density of 10^3 cells per well for 48 h before initiating the treatment. Treatments were added for another 72h. For Supplementary Fig. S2, Ishikawa, Hec50co and KLE cells were plated and treated as described in the main manuscript. For Supplementary Fig. S5, Hec50co cells were plated in 96 well plates at density of 10^3 cells per well for 48 h before initiating treatment. Cells were treated with either 5 nM PTX**s**, 5 nM PTX**p** (75/T), or Blank**p** (75/T) =5 nM for 24 h.

1.4 Assessment of NP uptake by blood-brain barrier (hCMEC/D3) cells

RHD**p** (75/T) were prepared using the nanoprecipitation method as described in section 3.3.1 of the main manuscript. Cell uptake was assessed using flow cytometry as mentioned in section 3.3.5 of the main manuscript. Briefly, blood-brain barrier cells (hCMEC/D3) were plated in 12 well plates at a density of 0.5×10^6 cells per well. After 24 h, RHD (0.01 µg) was added to each well in serum-free medium either in particulate form (RHD**p** (75/T)) or in soluble form (RHD**s**). Untreated cells served as the control. Six hours later, the cells were washed with PBS twice, trypsinized and quenched with serum containing media. Cells were then centrifuged at 230 ×g for 5 min, resuspended in 300 µL of fresh media and kept on ice until analysis was performed.

1.5 Determination of DNA content in Hec50co cells

The DNA content of Hec50co cells was assessed after treatment using CyQUANT® Direct Cell Proliferation Assay Kit (Thermo Fisher Scientific). Briefly, Hec50co cells were seeded into a 96 well plate at a density of 10^3 cells per well and incubated for 48 h. After incubation, cells were treated with 5 nM PTX**s**, 5 nM PTX**p** (75/T), or Blank**p** (75/T) =5 nM. Untreated cells served as the control. In order to ensure there were enough viable cells for the assay, the cells were treated for only 24 h. After treatment, the media was removed from the cells and replaced with 100 µL of fresh media and 100 µL of 2X detection reagent. The cells were incubated for 30 min before measuring the fluorescence at λex 480 and λem 535 using a SpectraMax M5 multimode microplate reader. The percent DNA content was calculated as the DNA content of each treatment group normalized to the DNA content of the control cells . The

fluorescence intensity of 100 µL of medium plus 100 µL of 2X detection reagent in the absence of cells was used as a blank and subtracted from all data.

1.6 Viable cell count using trypan blue in Hec50co cells

Hec50co cells were seeded into 100 mm cell culture dishes at a density of 0.5×10^6 cells per dish in 9 mL of medium. The cells were incubated for 48 h after which, 3 mL of each treatment was added. The treatment groups were the same as in the DNA content assay. After the cells were treated for 1 day, they were trypsinized and suspended in cell culture medium. The number of viable cells in each sample was determined using trypan blue staining (J.T.Baker Chemical Co., Philipsburg, NJ)

1.7 Determination of ATP content in Hec50co cells

ATP content was determined using the ATP Assay Kit (Abcam, Cambridge, MA). The same Hec50co cell samples used for the trypan blue assay were used in the ATP assay following the manufacturer's guidelines. Briefly, the cells were washed with cold PBS, resuspended in 100 µL of ATP buffer and homogenized by pipetting up and down. The insoluble material was pelleted by centrifuging at 13,000 xg for 5 minutes and the supernatant was transferred to a new tube. The samples were deproteinized using the Deproteinizing Sample Preparation Kit – TCA (Abcam) according to the manufacturer's protocol. After deproteinization, 50 µL of each sample was added to a 96-well plate along with 50 µL of the reaction mix. A standard curve was constructed alongside the unknown samples according to the provided protocol. After 30 min of incubation at room temperature, the fluorescence intensity was measured at λex 535 and λem 587 using SpectraMax M5 multimode microplate reader. The ATP content of unknown samples was determined using the standard curve and linear regression. Finally, the samples were normalized to the number of cells determined during the trypan blue assay.

1.8 Apoptosis assay with annexin V/propidium iodide staining in Hec50co cells

Cellular apoptosis of Hec50co cells was determined using the eBioscience™ Annexin V Apoptosis Detection Kit (Thermo Fisher Scientific). For this assay, the cells were seeded in 6-well plates at a density of 3×10^4 cells per well in 4.5 mL of medium and incubated for 48 h. Afterwards, 1.5 mL of each treatment was added to the wells (the same treatment groups as in the DNA, ATP and trypan blue assays). After 24 h of treatment, the cells were rinsed once with PBS and once with the 1X binding buffer provided in the kit. Then, the cells were resuspended in 1X binding buffer at a concentration of $1\text{-}5\times10^6$ cells/mL. Next, 5 µL of FITC-Annexin V was added to 100 µL of the cell suspension. The samples were incubated for 15 min at room temperature. After incubation, the cells were washed with 1X binding buffer and resuspended in 200 µL of 1X binding buffer. Five µL of propidium iodide (PI) staining solution was added to the cell suspensions. The samples were analyzed using flow cytometry. Data were gated as indicated in Supplementary Fig. S5 to determine the percentage of cells in apoptosis.

1.9 Bright field microscopic evaluation of Hec50co cells

Hec50co cells were grown in 6-well plates at a density of 3×0^4 cells per well in 4.5 mL of medium and incubated for 48 h. After incubation, 1.5 mL of each treatment was added to the wells (the same treatment groups as in the DNA, ATP, trypan blue and apoptosis assays). After 1 day, the cells were analyzed with 10× magnification using an Olympus inverted microscope (CKX41, Center Valley, PA). Images were acquired with an Olympus DP70 digital camera.

1.10 Quantitative estimation of PTX in murine tumors using LC-MS/MS

1.10.1 LC-MS/MS condition for PTX

A Shimadzu LC-MS/MS system (LC-MS/MS 8060, Shimadzu, Japan), LC system equipped with two pumps (LC-30 AD) and column oven (CTO-30AS) along with an auto-sampler (SIL-30AC) was used to inject 10 µL aliquots of the processed samples.

Mass spectrometric detection was performed on an 8060 mass spectrometer equipped with a DUIS source in positive mode. The MS/MS system was operated at unit resolution in the multiple reaction monitoring (MRM) mode, using precursor ion → product ion combinations of 854.30→286.15 m/z for PTX and 859.35→291.15 m/z for the internal standard (IS) (PTX-d5, Toronto Research Chemicals Inc., Toronto, ON, Canada). The compound-dependent mass spectrometer parameters, such as temperature, voltage, gas, and pressure, were optimized by auto method optimization via precursor ion search for each analyte and the internal standard (IS) using a 0.5 µg/mL solution in acetonitrile. PTX and PTX-d5 were detected in the positive ionization mode with the following instrument dependent mass spectrometer parameters: nebulizer gas: 2.0 L/min; heating gas: 10 L/min; drying gas: 10 L/min; interface temperature: 375 ºC; desolvation line temperature: 250 ºC; heat block temperature: 400 ºC and interface. UPLC and MS systems were controlled by LabSolutions LCMS Ver.5.6. (Shimadzu Scientific, Inc)

The compound PTX resolution and acceptable peak shape were achieved on a ACE Excel C18 (1.7µm, 2.1 X 100 mm, Advance Chromatography Technologies, LTD., UK) column protected with a C18 guard column (Phenomenex, Torrance CA). The PTX-d5 was used as the IS. The mobile phase consisted of 0.2% formic acid in water (mobile phase A) and acetonitrile (ACN) (mobile phase B), at a total flow rate of 0.25 mL/min. The chromatographic separation was achieved using 7 min gradient elution. The initial mobile phase composition was 50% B, gradually increased to 95% B over 5 min, then held constant at 95% B for 1.0 min, and finally brought back to initial condition of 500% B in 0.5 min followed by 1-min re-equilibration. The injection volume of all samples was 10 µL.

1.10.2 Stock, standard and quality control samples preparation

Stock solutions (1 mg/mL) of PTX, and PTX-d5 (IS) were made in acetonitrile. The calibration standard stocks of analytes were prepared by step-wise dilution of the stock solution in acetonitrile over the concentration range of 0.5–1000 ng/mL. Quality control samples (QCs) at four different concentrations were used: lower limit of quantification (LLOQ - 0.5 ng/mL), low quality control (LQC - 2 ng/mL), middle quality control (MQC - 200 ng/mL) and high quality control (HQC- 750 ng/mL). QCs were prepared separately in three replicates, independent of the calibration standards. The IS was diluted to 1000 ng/mL in acetonitrile for spiking into tumor samples.

1.10.3 Sample preparation

The plasma and tumor samples were processed using a solid phase extraction technique (SPE). The samples were prepared by spiking 10 µL of appropriate calibration stock into 200 µL of blank tumor homogenate, and 10 µL of the IS solution (1000 ng/mL) was added. Tumor was homogenized in water (1:4) and tumor samples were centrifuged for 5 min at 3500 rpm prior to loading to the SPE cartridge. The SPE was carried out using Agilent bond Elute C18, 50 mg 1 mL Cartridge (Agilent). Cartridges were conditioned with 1 mL acetonitrile and followed by 1 mL water. Tumor samples (200 µL) spiked with 10 µL spiking standard and 10 µL IS, were diluted to 0.8 mL with 0.1% formic acid (FA) and then loaded into the SPE cartridges. The cartridges were washed with 1 mL of aqueous 5% acetonitrile and 0.5% formic acid. Analytes were eluted with 2 mL of acetonitrile. The eluents were collected in glass tubes and evaporated to dryness under nitrogen in water bath set at 50°C. The dry residues were finally

reconstituted in 100 µL 0.1% formic acid: acetonitrile (50:50) and 10 µL supernatant injected onto the HPLC.

1.10.4 Method Validation

The developed LC-MS/MS method was validated as per US-FDA guidance with respect to selectivity, specificity, lower limit of quantification (LLOQ), accuracy, precision, and matrix effect[1].

The sensitivity of the method was determined from the signal-to-noise ratio (S/N) of the response of analyte in calibration standards. The S/N ratio should be greater than three for the limit of detection (LOD) and greater than 10 for the LLOQ. The calibration curves were established by plotting the peak area ratio (analyte/IS) versus concentration for all analytes.

Intra- and inter-day accuracy and precision were evaluated from replicate PTX (n=5) of QC samples containing analytes at different concentrations (LLOQ, LQC, MQC and HQC) prepared on the same day. The precision was calculated in terms of % relative standard deviation (%R.S.D.). The accuracy was expressed as % Bias. The criteria for acceptability of the data included accuracy within ± 15% standard deviation (S.D.) from the nominal values and a precision within ± 15% R.S.D. except for LLOQ, where it should not exceed ± 20% of accuracy as well as precision.

$$\%\text{Bias} = (\text{observed concentration} - \text{nominal concentration}) / \text{nominal concentration} \times 100$$

The carry-over was checked by injecting two zero samples directly after injecting an HQC sample. The response of the first zero sample should be < 20% of the response of a processed LLOQ sample.

The dilution effect was investigated to ensure that tumor homogenate samples could be diluted with water without affecting the result. Analytes spiked stripped serum prepared at 2000 ng/mL concentrations were diluted with stripped serum at dilution factors of 5 and 10 in five replicates and analyzed. As part of the validation, five replicates had to comply with both precision of ≤ 15% and accuracy of 100 ± 15% similar to other QCs samples.

The absolute recovery of PTX and IS were calculated by comparing the peak area of QC samples (LQC, MQC and HQC, n=3) in plasma with corresponding standard concentrations prepared in reconstitution solvent. The recovery was deemed acceptable if the % coefficient of variation (CV) was ± 20% among the mean recoveries at LQC and HQC levels.

1.11 Preparation of DiR-loaded PLGA NP

The near infrared (IR) fluorescent DIR dye (1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide, Invitrogen) was loaded in PLGA NP (DIR**p** (75/T)) to aid in tracking the biodistribution of these NP once administered intravenously. DIR**p** (75/T) were prepared by the nanoprecipitation method as described in section 3.3.1 of the main manuscript using 1 mg of DIR dye. The loading of DIR was determined by dissolving a known amount NP in DMSO. A standard curve was constructed from known concentrations of DIR dissolved in DMSO. Fluorescence detection was used to quantify the amount of DIR in the NP suspension at λex 750 and λem 780 using a SpectraMax M5 multimode microplate reader.

1.12 Biodistribution study

NCI-nu/nu mice were challenged with 2×10^6 Hec50co cells. Balb/c mice were challenged with either 3×10^6 CT26 cells or 5×10^6 A20 cells. Once the tumor size reached ~500 mm^3, the mice were IV injected (retro-orbital injections) with equal doses of DIR**p** (75/T) equivalent to 5 µg DIR. Forty-eight hours after

the injection of NP, the organs of the mice were harvested and analyzed using an IVIS-200 instrument (Xenogen, PerkinElmer, Waltham, MA) with an ICG filter. Images were analyzed using Living Image software by measuring the total flux from each organ. The baseline flux for each organ was determined from the control sample and was subtracted from all data. To determine percent total flux, the individual flux measurements from each organ of the mouse was summed. Then, the flux contribution from each organ was divided by the total flux from the summation of all organs and multiplied by 100 (see the equation below).

$$\text{Total Flux (\%)} = \frac{\text{Flux of individual organ}}{\text{Total flux of all organs summed together}} \times 100$$

2 Supplementary results and discussion for the LC-MS/MS quantification of PTX

2.1 Chromatographic and mass spectrometric conditions optimization

To obtain the selectivity and sensitivity for all analytes, several chromatographic and mass spectrometric conditions were optimized. The selection of the ionization mode was based on the comparison of obtained sensitivity with electro spray ionization (ESI) and atmospheric pressure chemical ionization (APCI) source. The results showed that ESI in positive mode could offer much higher intensity for the analytes than APCI (data not shown). The fragmentation of PTX and IS were auto optimized via precursor ion search of approximately 1000 ng/mL of stock solution of each analyte. The most abundant precursor > product ions in terms of better sensitivity for PTX and PTX-d5 at m/z 854.30→286.15 and 859.35→291.15 (Supplementary Fig. S7 a&b). These ions represented the fragmentation at the ester bond and a loss of the taxane structure. The compound dependent parameters such as voltage potential Q1 -26 and -28 (V) and Q3 -20 and -30 (V), collision energy (CE) -20 and -22, were also optimized to obtain the highest signal intensity for PTX and IS, respectively.

Chromatographic conditions, especially the composition of mobile phase and different analytical columns were optimized to achieve good resolution and symmetrical peak shapes of the analytes, as well as a short run time. The suitability and robustness of the method were evaluated using different varieties of reverse phase HPLC columns ranging from 50 to 150 mm in length (data not shown). Complete and rapid chromatographic resolution of analytes and IS was achieved on ACE Excel C18 column (1.7 µm, 100 x 2.1 mm) equipped with a C18 guard column. A better chromatogram with symmetrical peak shape was obtained using 0.2% FA and acetonitrile at a flow rate of 0.25 mL/min. with 40 °C as the column temperature. The representative overlay chromatograms with blank tumor homogenate in Supplementary Fig. S7 c&d show no interference of endogenous compounds at the retention time of PTX (3.2 min) and PTX-d5 (3.2 min) for samples spiked at 1.0 ng/mL concentration. The PTX-d5 was selected as the IS for PTX in this method. They had similar chromatographic behaviors and similar ionization responses in ESI mass spectrometry to that of analytes.

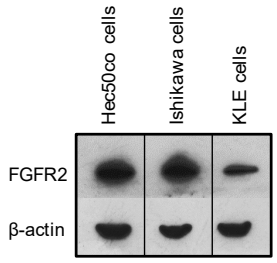
2.2 Method Validation

The method was validated for PTX using three calibration curves prepared on three days. The calibration curves were established by plotting the peak area ratio (peak area analyte/peak area IS) versus nominal concentration least-squares linear regression analysis with a weighting factor of $1/x^2$. The calibration curves were linear over the concentration range of 0.5 – 1000 ng/mL with a correlation coefficient $r^2 \geq 0.9980 \pm 0.0023$ (Supplementary Fig. S7 e&f). The intra-day inter-day accuracy and precision at five replicates of four different QCs (LLQC-QC, LQC, MQC and HQC) was found within acceptable 85-115% limits. A processed zero blank sample (Blank + IS) injected after ULOQ samples showed peak area < 5% of LLOQ resulting in no carry over effect.

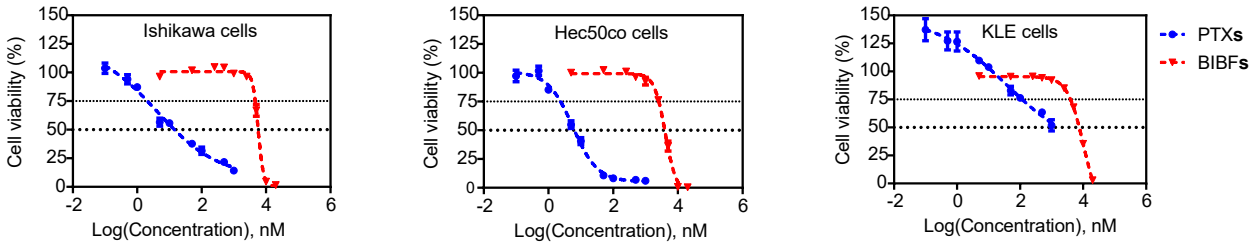
The precision for dilution integrity of 1:5 and 1:10 dilution were within acceptable limit for PTX, which is within the acceptance limits of ± 15% for precision (CV) and 85.0–115.0% for accuracy. The results suggested that plasma or tumor samples whose concentrations above upper limit of quantitation can be determined by appropriate dilution.

The % mean recovery was determined by measuring the response of the extracted plasma quality control samples at HQC, MQC and LQC against un-extracted quality control samples at HQC, MQC and LQC. The mean recovery of all three QC levels was 95.60%, whereas the mean recovery of IS was 90.71%.

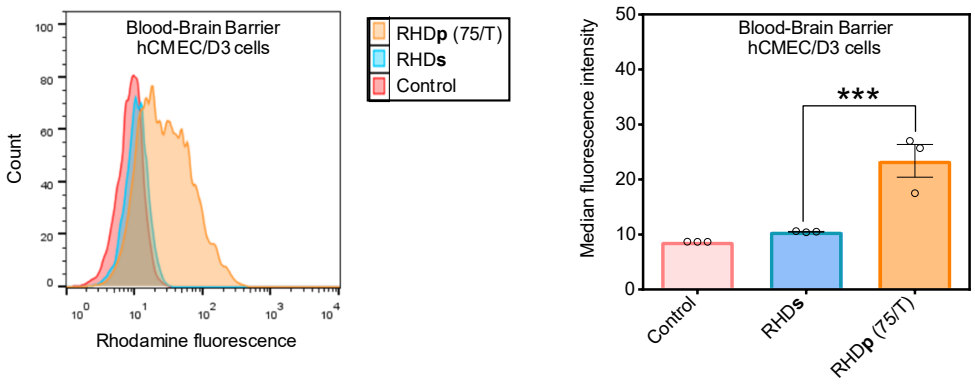
3 Supplementary figures



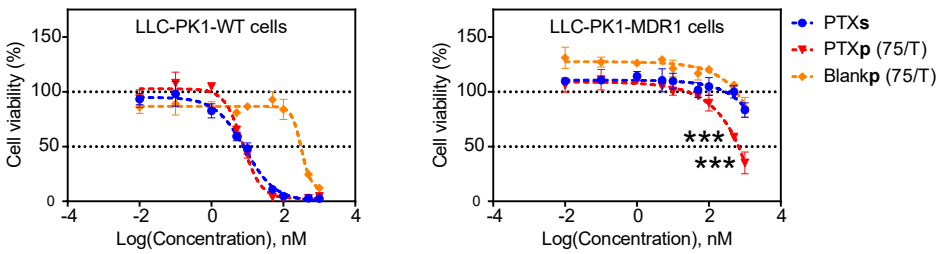
Supplementary Figure S1: BIBF target FGFR2 is expressed in three endometrial cancer cell lines: Hec50co, Ishikawa and KLE. Representative western blot depicting FGFR2 expression. β-actin, loading control. Cells were also screened for the presence of FGFR2 activating mutations, which occur in ~ 10-16% of endometrial cancers[2,3]. Previous reports have established that KLE and Ishikawa cells contain WT FGFR2. To confirm the published data and to determine if Hec50co cells contain WT or activated FGFR2, mutational hotspot regions in the third immunoglobulin domain (IIIC) and the transmembrane domain of FGFR2 were sequenced in the three cell lines. No mutations in FGFR2 were detected, indicating that all three cell lines contain WT FGFR2.



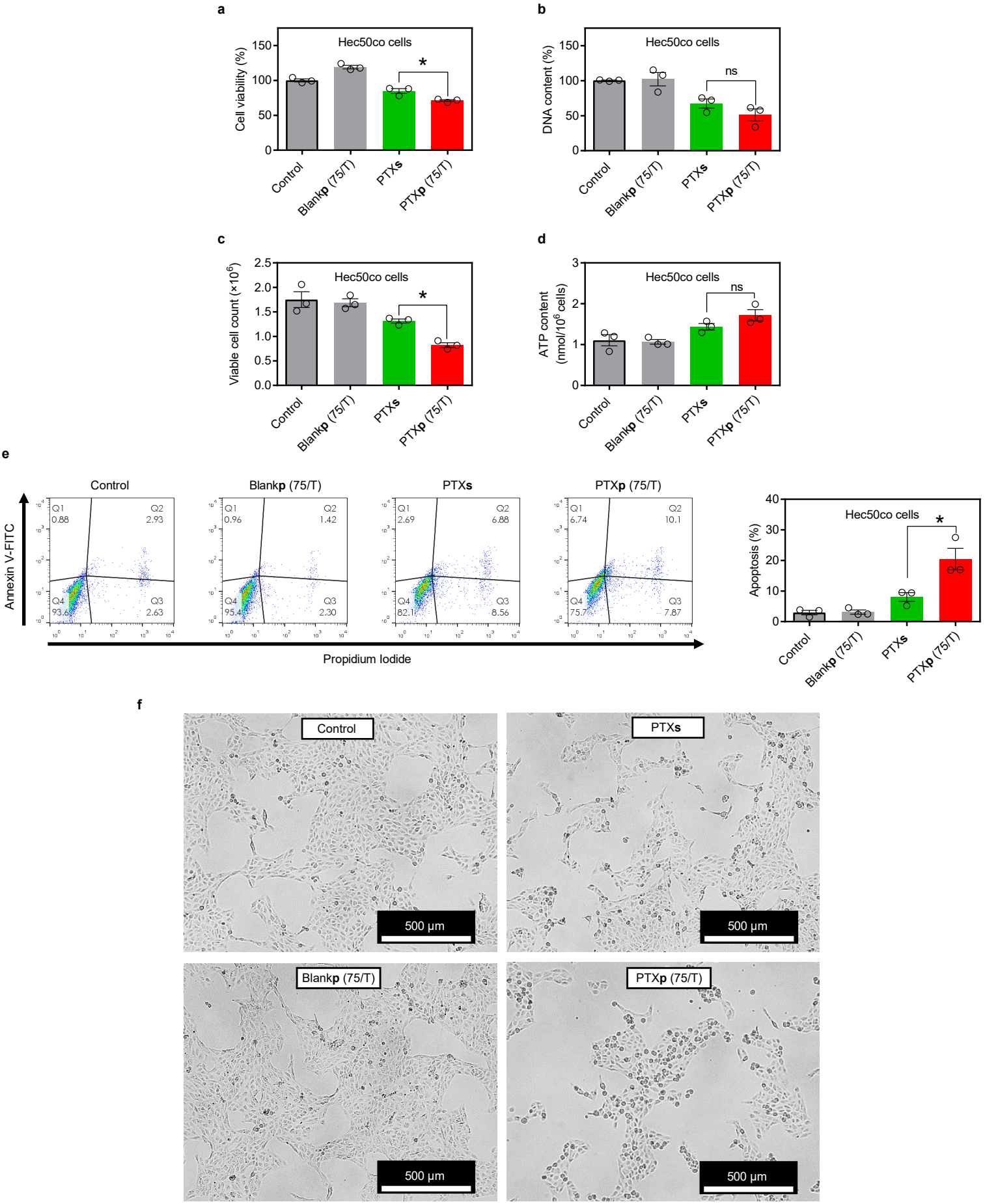
Supplementary Figure S2: Dose response curves of three EC cell lines. Indicated cells were incubated with soluble forms of either drug alone for 72 h, and cytotoxicity was evaluated using the MTS cell proliferation assay. Data are expressed as mean ± SEM (n=3).



Supplementary Figure S3: Significantly increased RHD uptake was observed when blood-brain barrier (hCMEC/D3) cells were treated with RHD**p** (75/T) versus RHD**s**. Cells were incubated with either 0.01 µg of RHD**s** or RHD**p** (75/T) for 6 h in serum free media, and then uptake was evaluated using flow cytometry. Left, representative histograms of different treatments. Right, bar chart summarizing the median fluorescence intensity of each treatment. Statistical analysis was performed using one-way ANOVA with Tukey post hoc test. Data are expressed as mean ± SEM (n=3). *** $p<0.001$.



Supplementary Figure S4: PTX**p** (75/T) was significantly more cytotoxic than PTX**s** against the PTX-resistant cell line, LLC-PK1-MDR1. Cells were incubated with different concentrations of PTX**s**, PTX**p** (75/T), Blank**p** (75/T) for 72 h, and cytotoxicity was evaluated using the MTS cell proliferation assay. Statistical analysis was performed using one-way ANOVA with Tukey post hoc test. Data are expressed as mean ± SEM (n=3). *** $p<0.001$.


a
Hec50co cells
Cell viability (%)
150
100
50
0
*
Control
Blankp (75/T)
PTXs
PTXp (75/T)
b
Hec50co cells
DNA content (%)
150
100
50
0
ns
Control
Blankp (75/T)
PTXs
PTXp (75/T)
c
Hec50co cells
Viable cell count (×10^6)
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
Blankp (75/T)
PTXs
PTXp (75/T)
d
Hec50co cells
ATP content (nmol/10^6 cells)
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2
1
0
ns
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PTXs
PTXp (75/T)
e
Control
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PTXs
PTXp (75/T)
Annexin V-FITC
Propidium Iodide
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Q2 2.93
Q4 93.6
Q3 2.63
Q1 0.96
Q2 1.42
Q4 95.4
Q3 2.30
Q1 2.69
Q2 6.88
Q4 82.1
Q3 8.56
Q1 6.74
Q2 10.1
Q4 75.7
Q3 7.87
Hec50co cells
Apoptosis (%)
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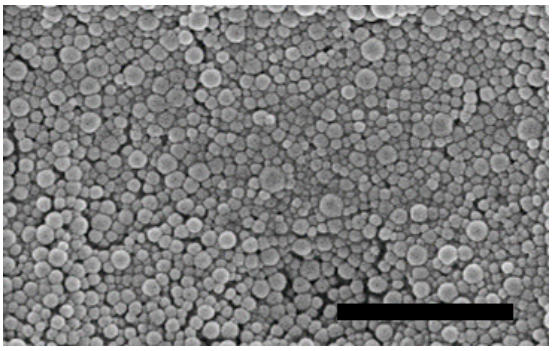
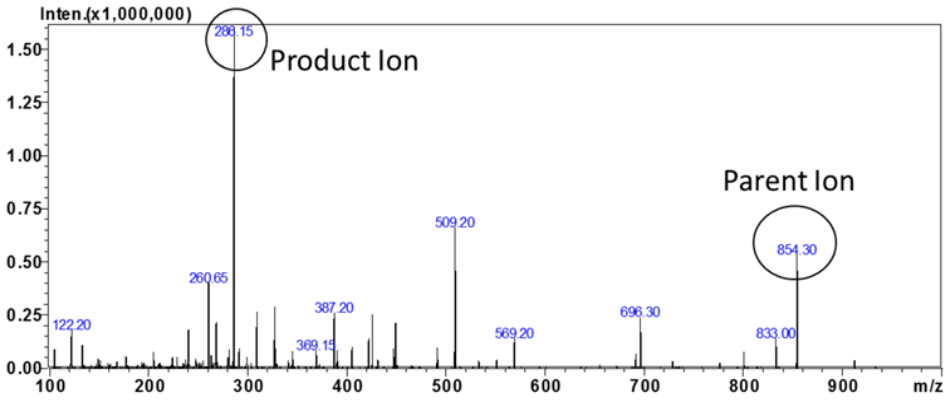
30
20
10
0
*
Control
Blankp (75/T)
PTXs
PTXp (75/T)
f
Control
PTXs
Blankp (75/T)
PTXp (75/T)
500 μm
500 μm
500 μm
500 μm

Supplementary Figure S5: PTX**p** (75/T)-induced cytotoxicity against Hec50co cells is demonstrated by inhibition of cell proliferation, decreased DNA content, decreased number of viable cells, increased cellular ATP content, increased apoptosis, and increased cells undergoing mitosis. In this set of experiments, cells were incubated with either 5 nM PTX**s**, 5 nM PTX**p** (75/T), or Blank**p** (75/T) =5 nM for 24 h only, in order to maintain sufficient live cells to effectively perform each assay. **(a)** Cell viability was assessed using the MTS cell proliferation assay. **(b)** DNA content was estimated using the CyQUANT® direct cell proliferation assay. **(c)** Viable cell count was evaluated using trypan blue staining. **(d)** ATP content was estimated using the ATP assay kit. **(e)** Apoptosis (%) was evaluated using flow cytometry after staining the cells with Annexin V/PI (left panel), and the total percentage of cells in early and late apoptosis was calculated by summing the (%) of cells in both Q1 and Q2 (right panel). **(f)** Cells undergoing mitosis (rounded cells) were imaged using bright field microscopy utilizing 10x lens. Scale bar= 500 μm. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. Data are expressed as mean ± SEM (n=3). * $p<0.05$.



Supplementary Figure S6: Scanning electron micrograph of BIBF**p** (75/T) showing spherical nanoparticles with smooth surfaces. Scale bar = 1 μm.

a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Parent Ion
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696.30
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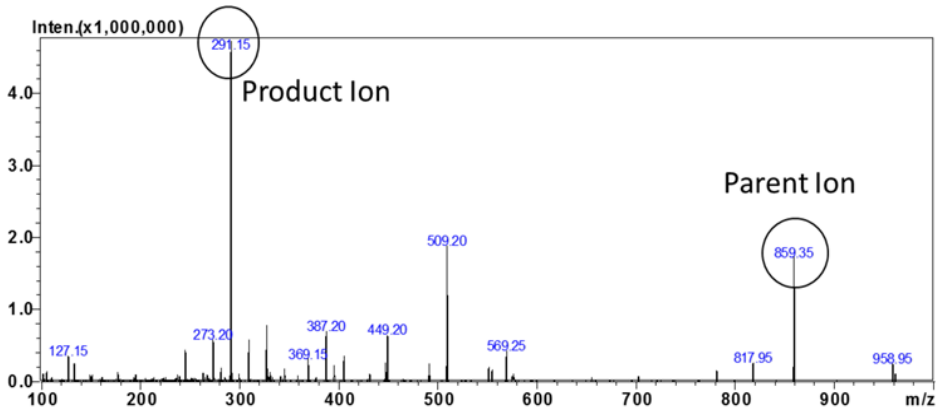
369.15
m/z

b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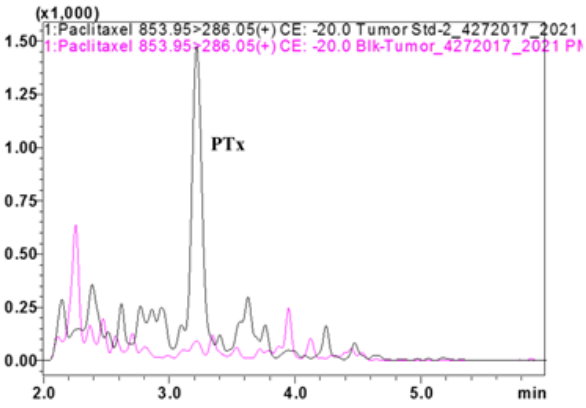
m/z

c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PTx
min

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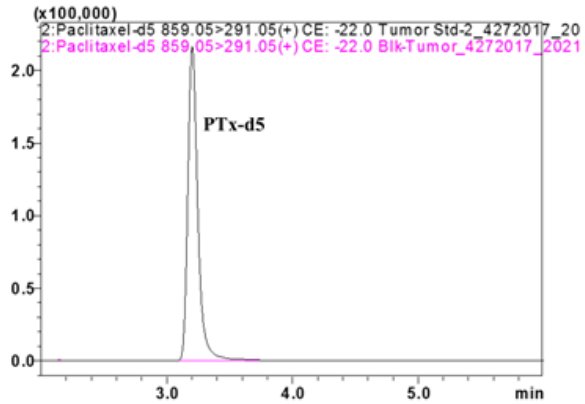
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PTx-d5
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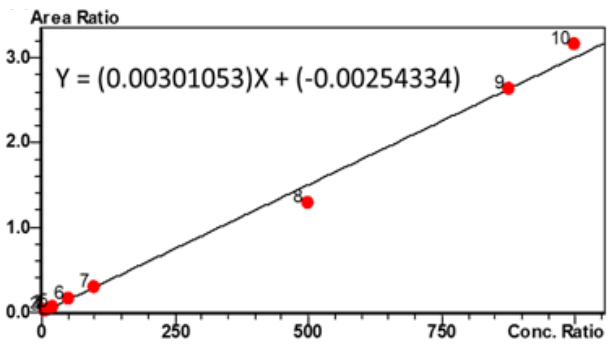
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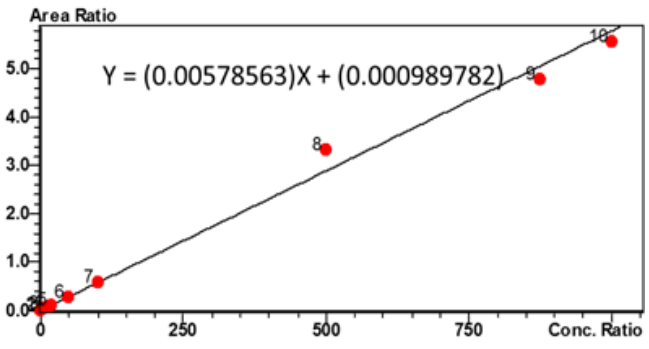
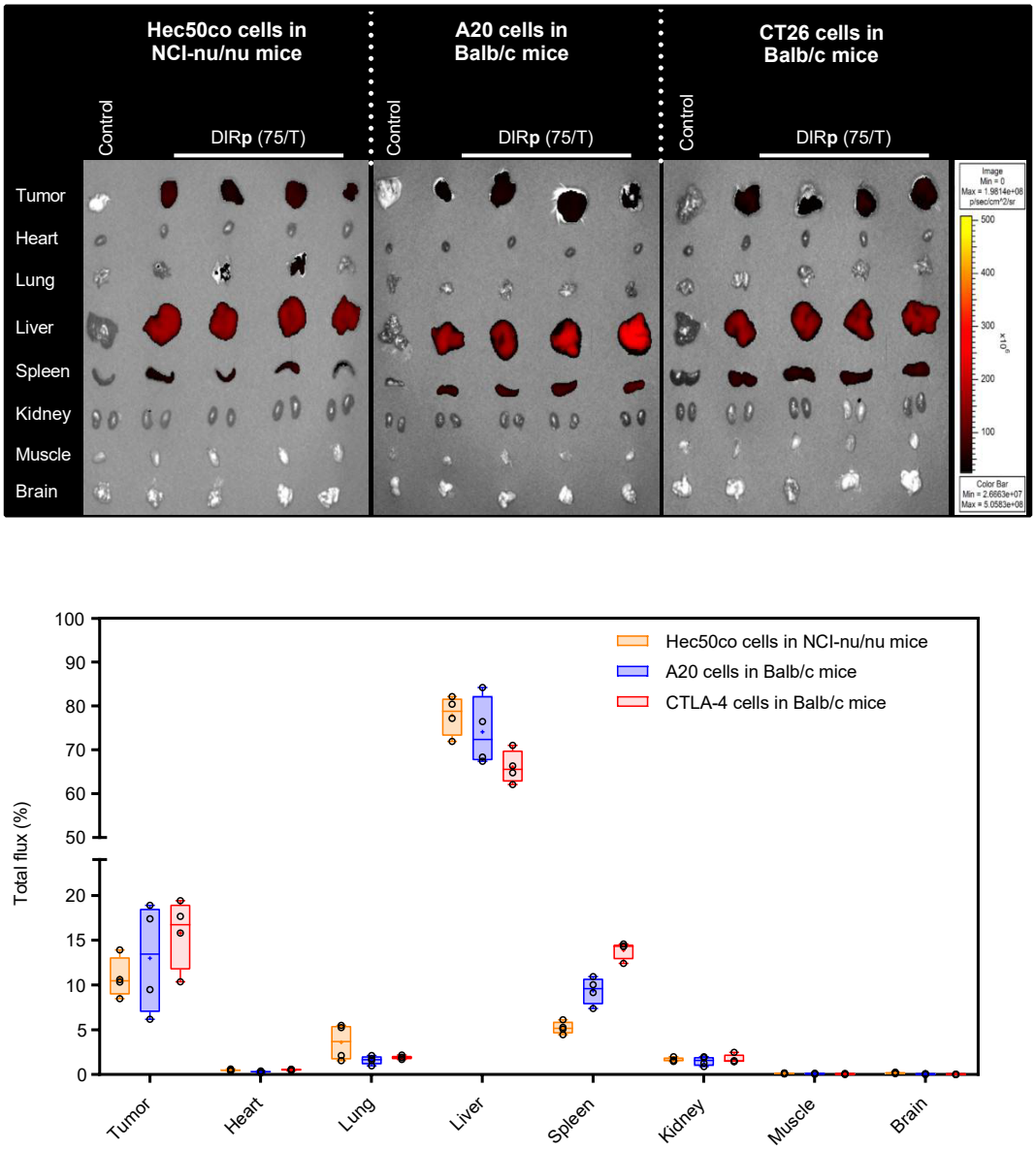
Conc. Ratio

f


Area Ratio
Y = (0.00578563)X + (0.000989782)
Conc. Ratio

Supplementary Figure S7: LC-MS/MS method validation for intra-tumoral PTX quantification **(a)** MS/MS spectra of PTX and fragmentation pattern of PTX with product ions m/z 696.30,569.20, 509.20,387.20 and 286.15, **(b)** MS/MS spectra of PTX-d5 (IS) with product ions m/z 569.20, 509.20,387.20 and 291.15. **(c & d)** Representative MRM ion- overlay chromatograms of **(c)** blank tumor homogenate and standard spiked PTX at 1.0 ng/mL, and **(d)** blank tumor homogenate and IS spiked PTX-d5 at 100 ng/mL. **(e & f)** Calibration curves in **(e)** neat solution and **(f)** tumor homogenate.



Supplementary Figure S8: Between 10-15% of the total DIR**p** (75/T) dose accumulated in the tumors of mice 48 h post IV injection. The biodistribution of DIR**p** (75/T) was assessed in three different murine tumor models. The top panel shows the IVIS fluorescence images of DIR**p** (75/T) in the organs of mice 48 h post injection. In each tumor model, an untreated mouse served as the control. The bottom panel shows a summary of fluorescence intensities of each organ normalized to the total fluorescence intensity of all organs (see methods and materials for details) in the various tumor models (n=4).

4 Supplementary references

1 Food and Drug Administration Centre for Drug Evaluation and Research (FDA). Guidance for Industry-Bioanalytical Method Validation. Silver Spring, MD: Center for Drug Evaluation and Research, US Department for Health and Human Services, May 2001. 2013.

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