Supplementary Information

Lysosome-Targeted Bioprobes for Sequential Cell Tracking from Macroscopic to Microscopic Scales

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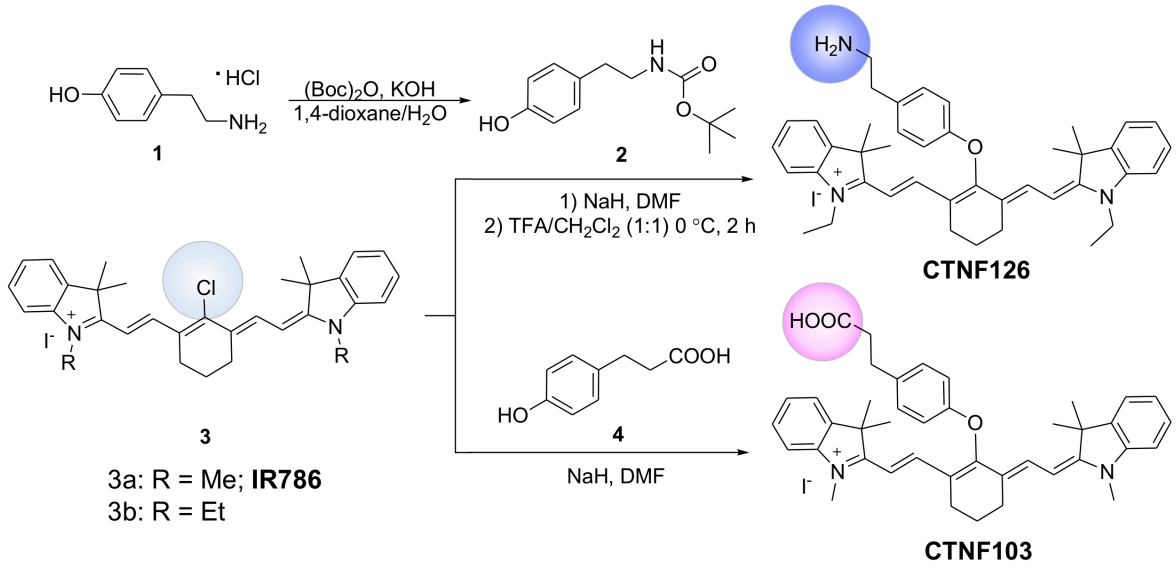
Supplementary Methods

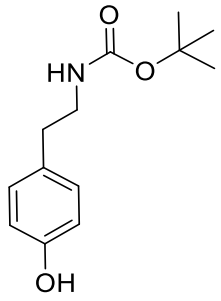
Materials: All chemicals and solvents were of American Chemical Society grade or HPLC purity and were used as received. All chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA), Sigma-Aldrich (Saint Louis, MO), and Acros Organics. The reactions were followed using silica gel 60 F254 thin layer chromatography plates (Merck EMD Millipore, Darmstadt, Germany). Column chromatography was utilized for the purification of all hydrophobic final compounds using 60−200 μm, 60A classic column silica gel (Dynamic Adsorbents, Norcross, GA). The ^{1}H NMR and ^{13}C NMR spectra were obtained using high quality Kontes NMR tubes (Kimble Chase, Vineland, NJ) rated to 500 MHz and were recorded on a Bruker Avance (400 MHz) spectrometer using $CDCl_3$, DMSO-d_6, or MeOD-d_4 containing tetramethylsilane (TMS) as an internal calibration standard set to 0.0 ppm. NMR abbreviations used throughout the experimental section are as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, dd = doublet doublets, and bs = broad singlet. High resolution mass spectra (HRMS) were obtained at the GSU Mass Spectrometry Facility using a Waters Q-TOF micro (ESI-Q-TOF) mass spectrometer. The purity of each compound tested was determined by using LC/MS instrument possessing a Waters 2487 single wavelength absorption detector. The column used in LC was a Waters Delta-Pak 5 μM 100Å 3.9×150 mm reversed phase C_{18} column, with a flow rate of 1 mL/min employing a 5-100% acetonitrile/water/0.1% formic acid gradient; a SEDEX 75 Evaporative light scattering detection (ELSD) was also utilized in tandem with liquid chromatography to confirm purity.

Synthesis and characterization of CTNF126 and CTNF103: Chloro substituted dyes **3a-b** were synthesized as previously reported through salt condensation with Vilsmeier-Haack reagent to build the heptamethine cyanine core.[1] The desired dyes shown in Scheme 1 were synthesized by reacting the meso chlorine atom of **3a-b** with the appropriate nucleophiles through the $S_{RN}1$ displacement pathway. Because the protonated oxygen exhibits limited nucleophilicity, NaH is used to generate the phenoxide ion in situ. For the amine containing compound, tyramine hydrochloride **1** was first Boc-protected to form compound **2** and ensure selective reactivity at the oxygen atom. The Boc-protected product is then converted to the free amine by treatment with trifluoroacetic acid (TFA) in dichloromethane at 0 °C to yield dye CTNF126. Final dyes **CTNF126** and **CTNF103** were then purified by flash column chromatography.



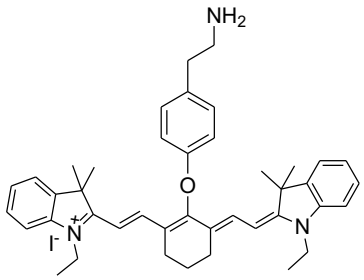
Scheme S1. Synthetic scheme for CTNF126 and CTNF103.

Tert-butyl 4-hydroxyphenethylcarbamate (**Compound 2**):



KOH (1.0 equiv) was added to a solution of tyramine hydrochloride **1** (831 mg, 6.07 mmol) in a mixture of dioxane/water (9.0/3.0 mL, 0.5 M). Di-*tert*-butyl dicarbonate (1.4 g, 6.23 mmol, 1.0 equiv) was then added, and stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo, and the residue was partitioned between dichloromethane (DCM) and 5% hydrochloric acid. The aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to afford pale yellow oil. The oil was purified by flash chromatography using hexane and ethyl acetate (50:50) to yield 837 mg (90%) **2** as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.47 (s, 9H), 2.74 (t, J = 6.8 Hz, 2 H), 3.35 (t, J = 6.8 Hz, 2 H), 4.68 (brs, 1H), 6.55 (brs, 1H), 6.80 (d, J = 8.3 Hz, 2 H), 7.02 (d, J = 8.3 Hz, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 28.4, 35.2, 41.9, 79.7, 115.5, 129.8, 130.4, 154.7, 156.5.

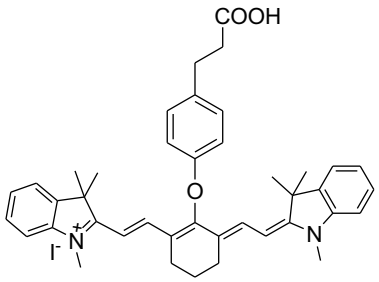
2-(4-(((E)-2-((E)-2-(1-ethyl-3,3-dimethyl-3H-1l4-indol-2-yl)vinyl)-6-(2-((E)-1-ethyl-3,3-dimethylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)oxy)phenyl)ethan-1-amine iodide (**CTNF126)**:



CTNF126

Under nitrogen, a solution of the Boc-protected tyramine **2** (237 mg, 1 mmol) in anhydrous DMF was added to NaH (21 mg, 0.8 mmol) in anhydrous DMF at 0 °C dropwise. After stirring the reaction mixture at 0 °C for 30 min, **3b** (210 mg, 0.3 mmol) in anhydrous DMF solution was added dropwise over 30 min and stirred for another 90 min at room temperature. After completion, the reaction mixture was quenched with ammonium chloride solution and extracted with DCM (3 × 100 mL). Combined organic extracts were dried over $MgSO_4$ and solvent was removed under vacuum, the crude product was dissolved in DCM and 3 mL of trifluoroacetic acid (TFA) was added at 0 °C dropwise. The reaction mixture was stirred at room temperature for 3h. After completion of the reaction, The vacuum removal of the solvent and TFA followed, and the obtained crude product was purified by column chromatography. 239 mg, 92% yield, mp. 218 - 221 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 1.12 – 1.30 (m, 18 H), 1.90 – 1.99 (m, 2 H), 2.70 – 2.75 (m, 4 H), 2.77 – 2.97 (m, 4 H), 4.20 (q, J = 6.5 Hz, 4 H), 6.20 (d, J = 14.1 Hz,2 H), 7.10 – 7.16 (m, 1 H), 7.19 – 7.25 (m, 1 H), 7.28 (d, J = 8.3 Hz, 1 H), 7.37 – 7.41 (m, 4 H), 7.48 – 7.53 (d, J = 7.5 Hz, 2 H), 7.82 (d, J = 14.1 Hz, 2 H), 7.99 (s, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 11.5, 20.1, 23.2, 27.6, 34.0, 41.3, 48.0, 99.2, 110.4, 133.7, 120.7, 121.9, 124.2, 127.9, 129.8, 122.7, 140.4, 141.1, 145.3, 157.5, 162.3, 170.5. HRMS: calcd for $C_{42}H_{50}N_3O^+$ m/z 612.3949, obsd 612.3951; calcd for $C_{42}H_{51}N_3O^{2+}$ m/z 306.7011, obsd 306.7009.

2-((E)-2-((E)-2-(4-(2-carboxyethyl)phenoxy)-3-(2-((E)-1,3,3-trimethylindolin-2-ylidene)-ethylidene)cyclohex-1-en-1-yl)vinyl)-1,3,3-trimethyl-3H-indol-1-ium iodide (**CTNF103)**:



CTNF103

Under nitrogen, a solution of the 4-hydroxyphenylpropionic acid **4** (271 mg, 1 mmol) in anhydrous DMF was added to NaH (61 mg, 2 mmol) in anhydrous DMF at 0 °C dropwise. After stirring the reaction mixture at 0 °C for 30 min, **3a** (256 mg, 0.4 mmol) in anhydrous DMF solution was added dropwise over 30 min and stirred for another 60 min at room temperature. The crude product was then precipitated in ether and purified by column chromatography. 252 mg, 81% yield, mp. 161 – 163 °C. ^{1}H NMR (400 MHz, MeOD-*d*$_4$): δ ppm 1.35 (s, 12 H), 2.05 (m, 2 H), 2.52 (t, *J* = 7.2 Hz, 2 H), 2.75 (t, *J* = 6.0 Hz, 4 H), 2.86 (t, *J* = 7.2 Hz, 2 H), 3.60 (s, 6 H), 6.13 (d, *J* = 14.4 Hz, 2 H), 7.03 (d, *J* = 8.8 Hz, 2 H), 7.25 (m, 6 H), 7.38 (m, 4 H), 8.00 (d, *J* = 14.4 Hz, 2 H). ^{13}C NMR (100 MHz, MeOD-*d*$_4$): δ ppm 21.0, 23.8, 26.6, 29.3, 29.8, 30.1, 36.0, 48.7, 99.5, 110.2, 114.3, 121.7, 121.8, 124.6, 128.2, 129.9, 135.3, 141.0, 142.0, 142.8, 158.5, 164.0, 172.9. HRMS: calcd for $C_{41}H_{45}N_2O_3^+$ m/z 613.3425, obsd 613.3430.

References

(1) Strekowski, L.; Lipowska, M.; Patonay, G. J Org Chem 1992, 57, 4578.

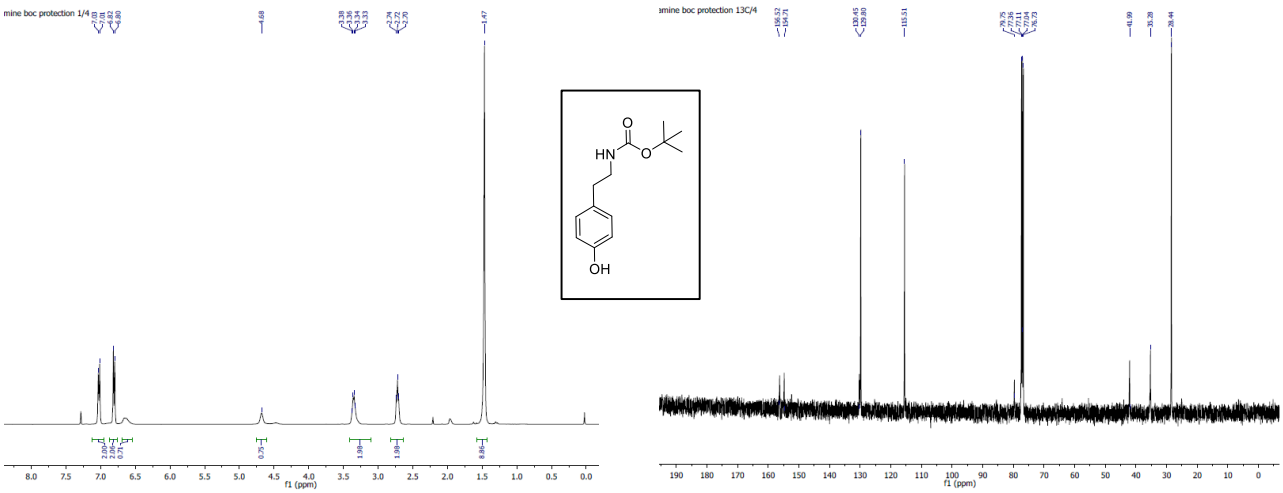


Figure S1. ^{1}H and ^{13}C NMR spectra of Compound 2.

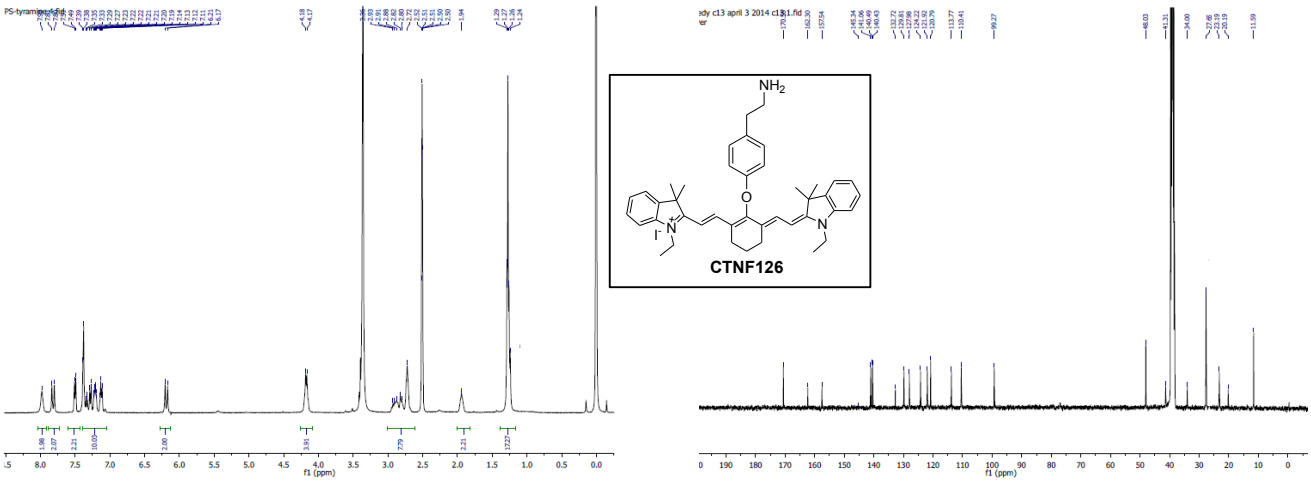


Figure S2. ^{1}H and ^{13}C NMR spectra of CTNF126.

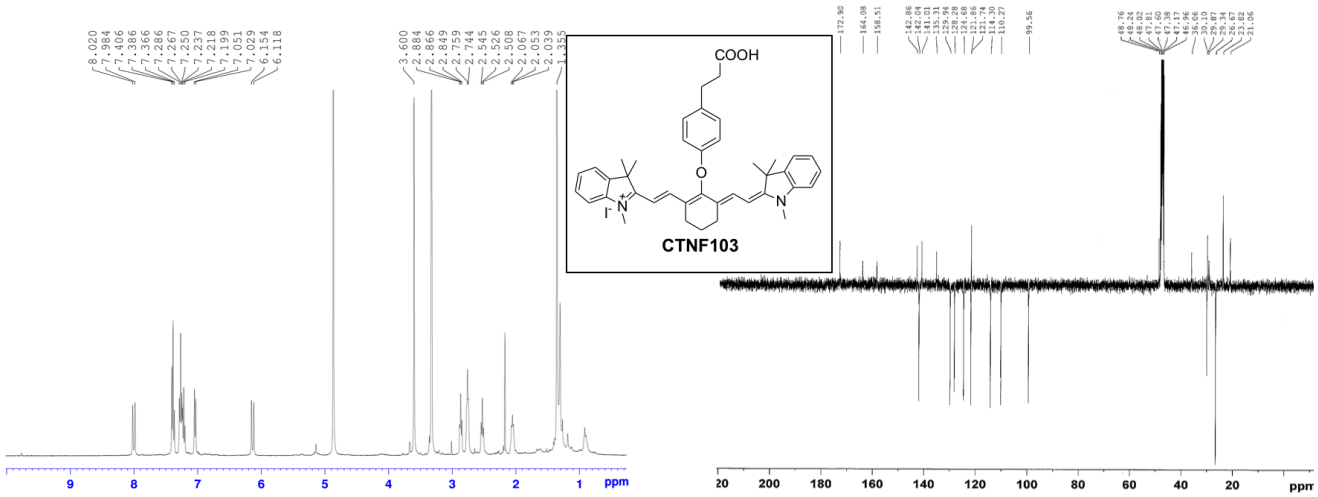


Figure S3. ^{1}H and ^{13}C NMR spectra of CTNF103.

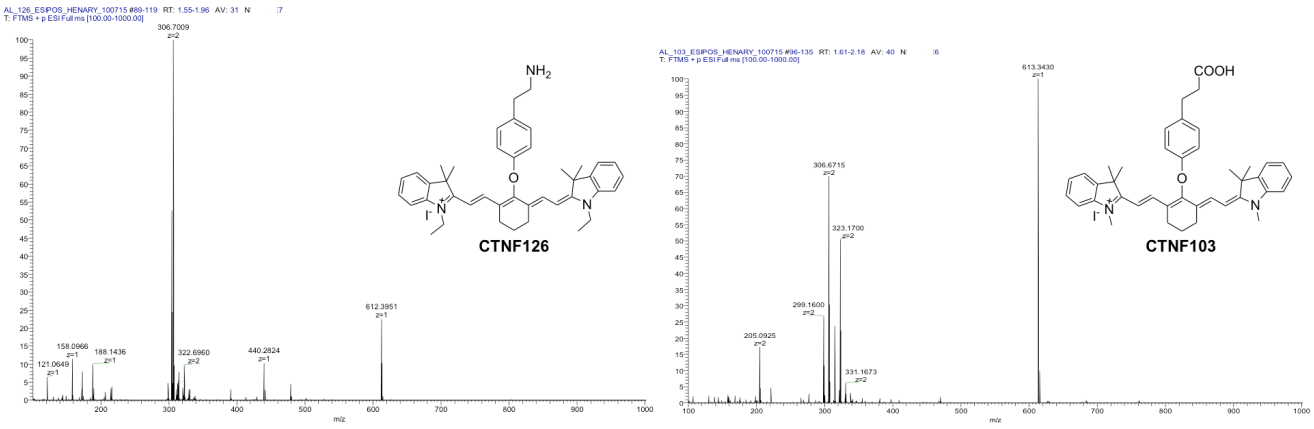


Figure S4. HPLC-MS analysis for CTNF126 and CTNF103.

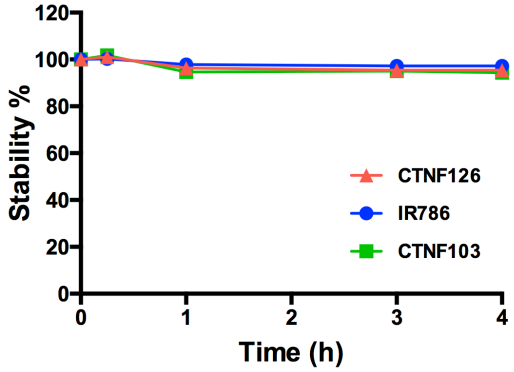


Figure S5. Photostability and physicochemical stability of CTNF126, IR786, and CTNF103. 5 μM of each fluorophore was incubated in fetal bovine serum (FBS) supplemented with 50 mM HEPES (pH 7.4) at 37 °C for 4 h.

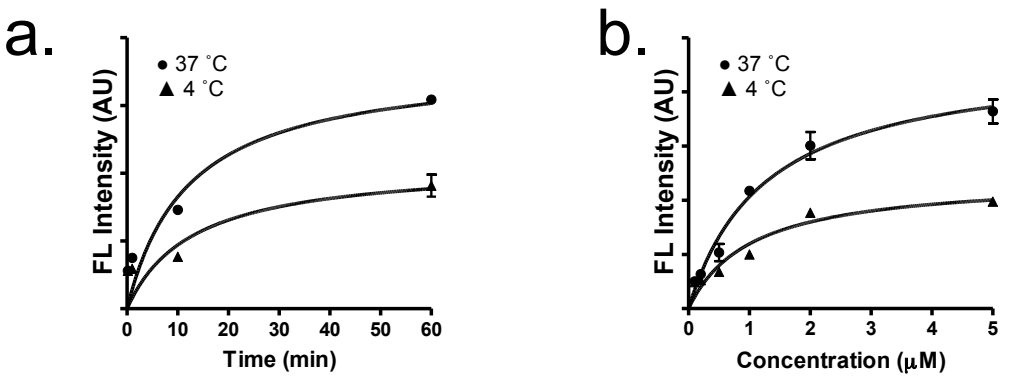


Figure S6. *In vitro* cellular uptake of CTNF126 in PC3 cells with various incubation times (**a**) and concentrations (**b**).

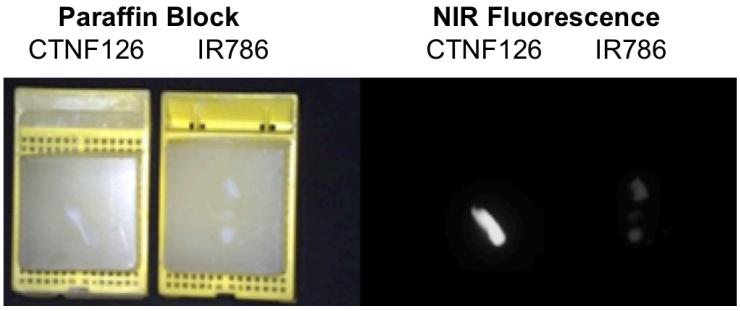


Figure S7. Thermal stability of CTNF126 and IR786. Each NIR fluorophore-labeled PC3 cell pellets were processed into a paraffin block and imaged under the NIR imaging system.

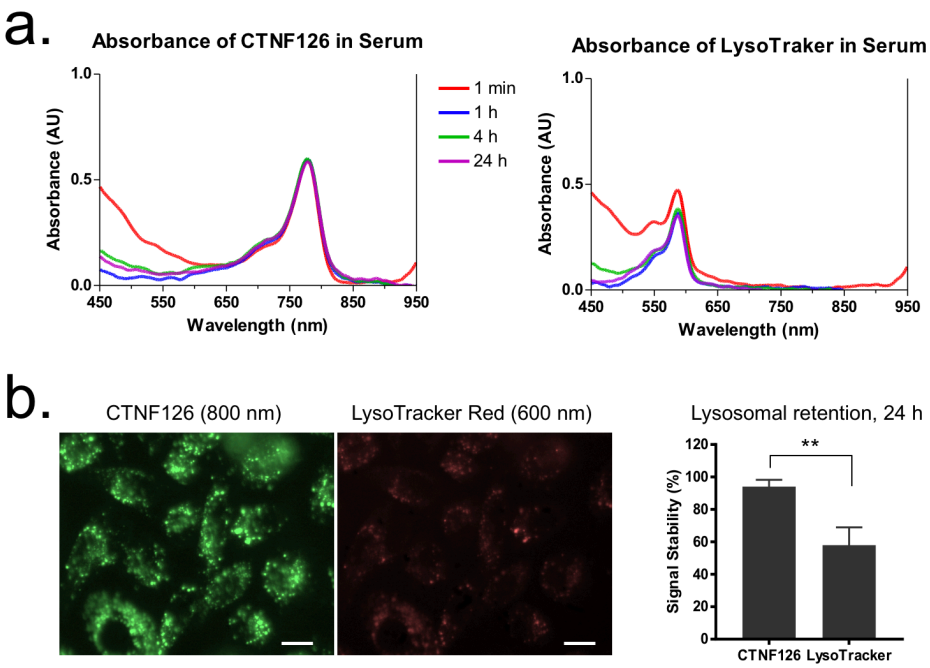


Figure S8. Biological stability of CTNF126 and LysoTracker. (a) Serum stability of CTNF126 (left) and LysoTracker (right) was measured in growth media at 37 °C for 1 - 24 h post-incubation. (b) Lysosomal retention of CTNF126 (left) vs. LysoTracker (right) in PC3 cells at 24 h post-incubation (n = 5, mean ± s.d., $^{**}P$ <0.01). Scale bar = 10 µm.