*Supporting information*

# Dual turn-on fluorescence signal-based controlled release system for real-time monitoring of drug release dynamics in living cells and tumor tissues

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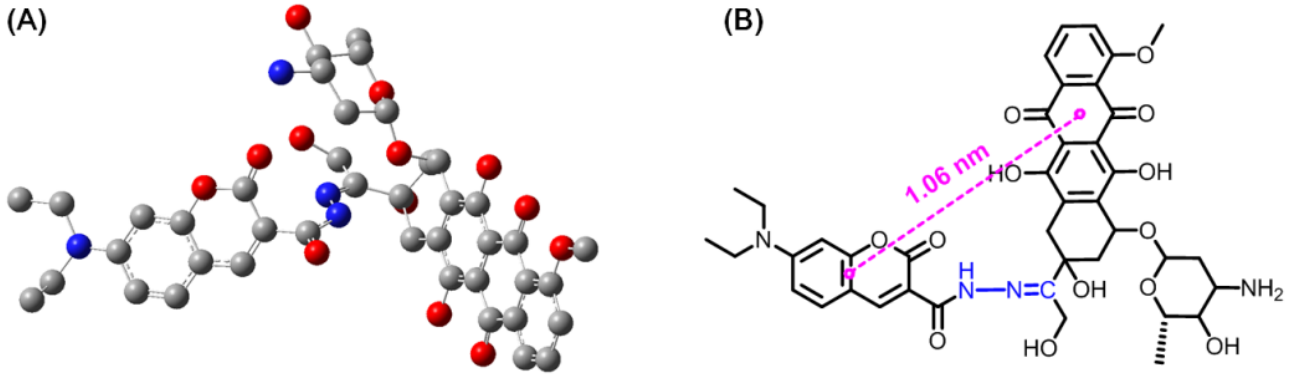


**Figure S1**. (A) DFT optimized structure of **CDox**. In the ball-and-stick representation, carbon, nitrogen, and oxygen atoms are colored in gray, blue, and red, respectively. H atoms were omitted for clarity. (B) DFT calculated distance of between **Dox** and **CH** moieties in **CDox**.

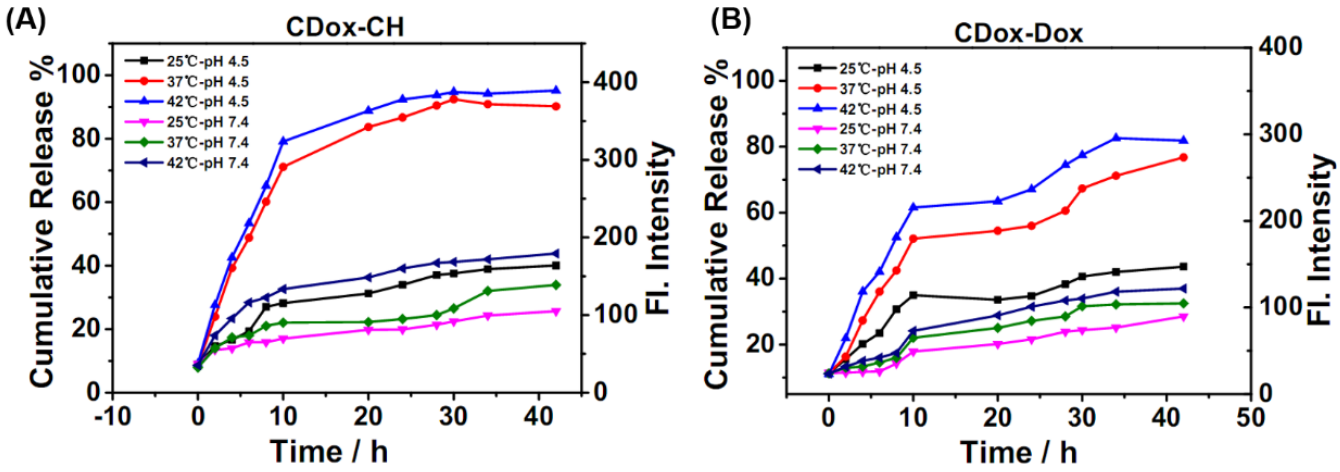


**Figure S2**. The temperature optimization experiments of the release of **CDox**. (A) The cumulative release curve of released **CH** from 2 μM **CDox** in pH 4.5 and 7.4 B-R buffer (10% DMSO) at 25°C, 37°C and 42°C respectively. (B) The cumulative release curve of released **Dox** from 2 μM **CDox** in pH 4.5 and 7.4 B-R buffer (10% DMSO) at 25°C, 37°C and 42°C respectively.

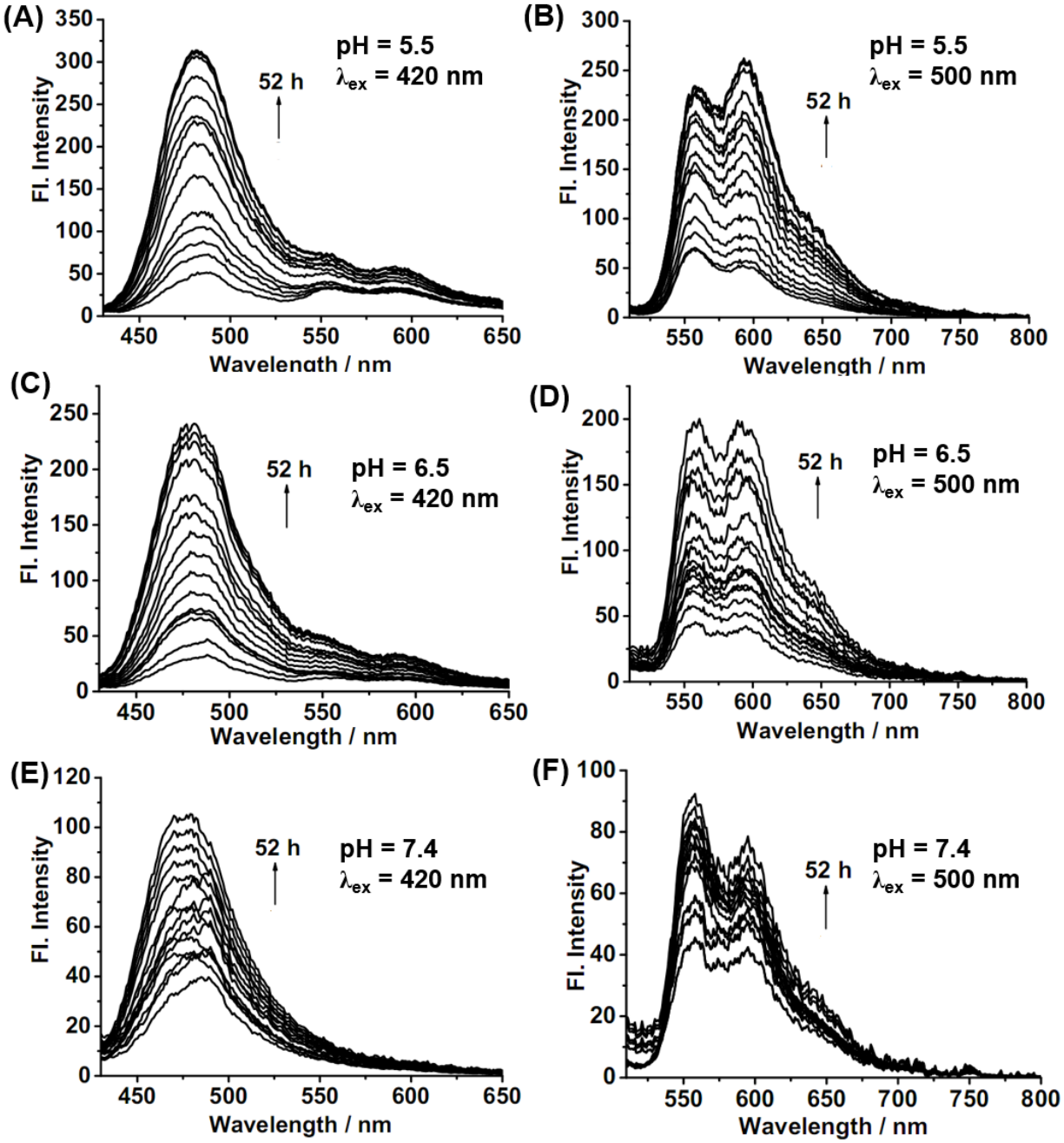


**Figure S3.** Time-dependent fluorescence spectra of 2 μM **CDox** in B-R buffer (10% DMSO) with various pH (pH 5.5, pH 6.5 and pH 7.4) under the excitation at 420 nm or 500 nm.

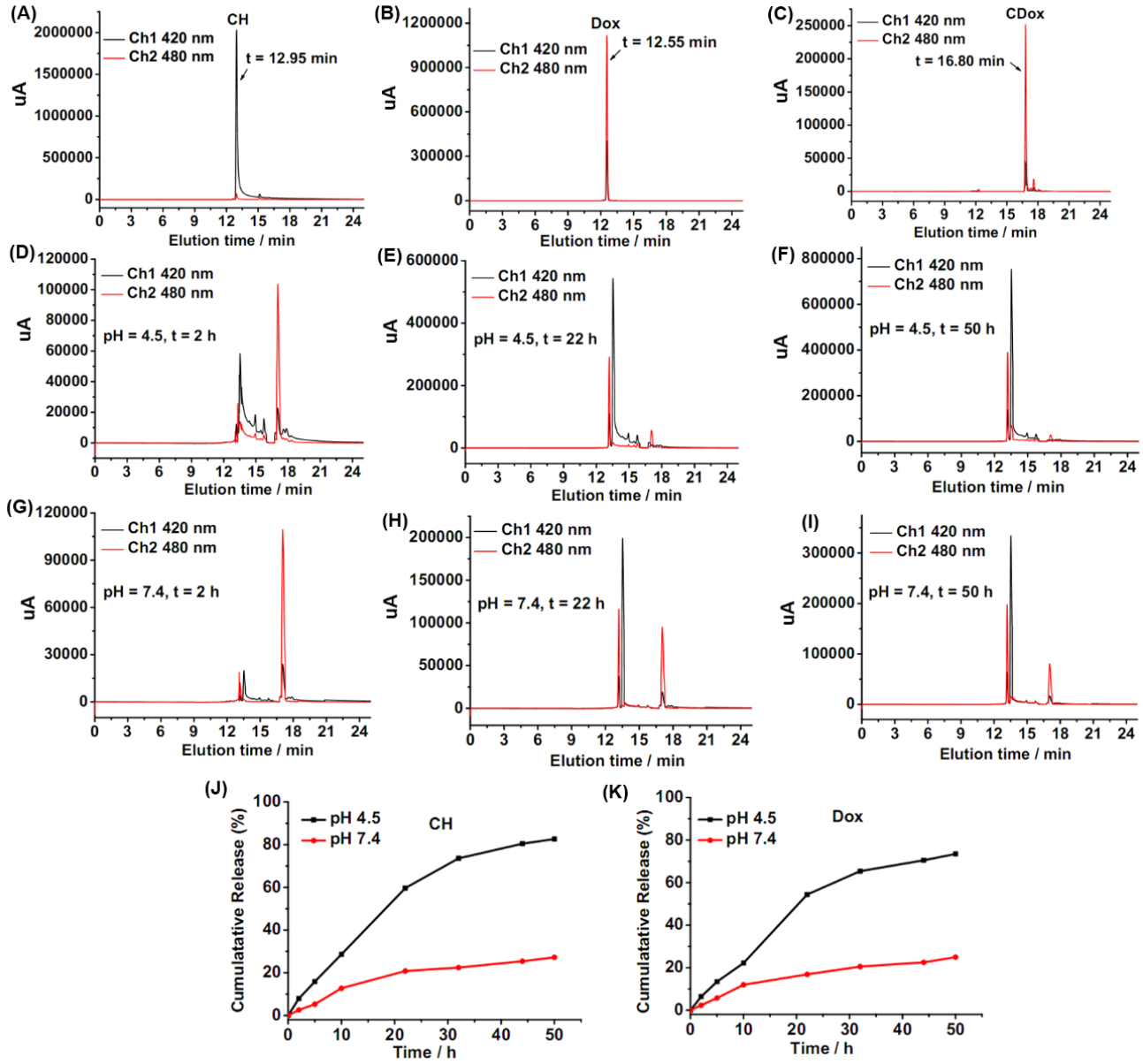


**Figure S4.** Typical HPLC data for 1mg/mL of free **CH** (A), **Dox** (B) and **CDox** (C) respectively. Representative HPLC chromatogram for the release of **CDox** (0.5 mg/mL) in pH 4.5 B-R buffer at 2 h (D), 22 h (E) and 50 h (F). Representative HPLC chromatogram for the release of **CDox** (0.5 mg/mL) in pH 7.4 B-R buffer at 2 h (G), 22 h (H) and 50 h (I). (J) Percentage cumulative release of released **CH** from **CDox** (as determined by HPLC) in pH 4.5 and pH 7.4 B-R buffer. (K) Percentage cumulative release of released **Dox** from **CDox** (as determined by HPLC) in pH 4.5 and pH 7.4 B-R buffer. Peaks in the chromatograms were detected by monitoring the absorption at 420 nm and 480 nm. The mobile phase was acetonitrile/water at a flow rate of 1.0 mL/min. Elution program: gradient from 5% to 95% of acetonitrile in 15 min, maintaining to 5 min and decrease from 95% to 5% of acetonitrile in 5 min.

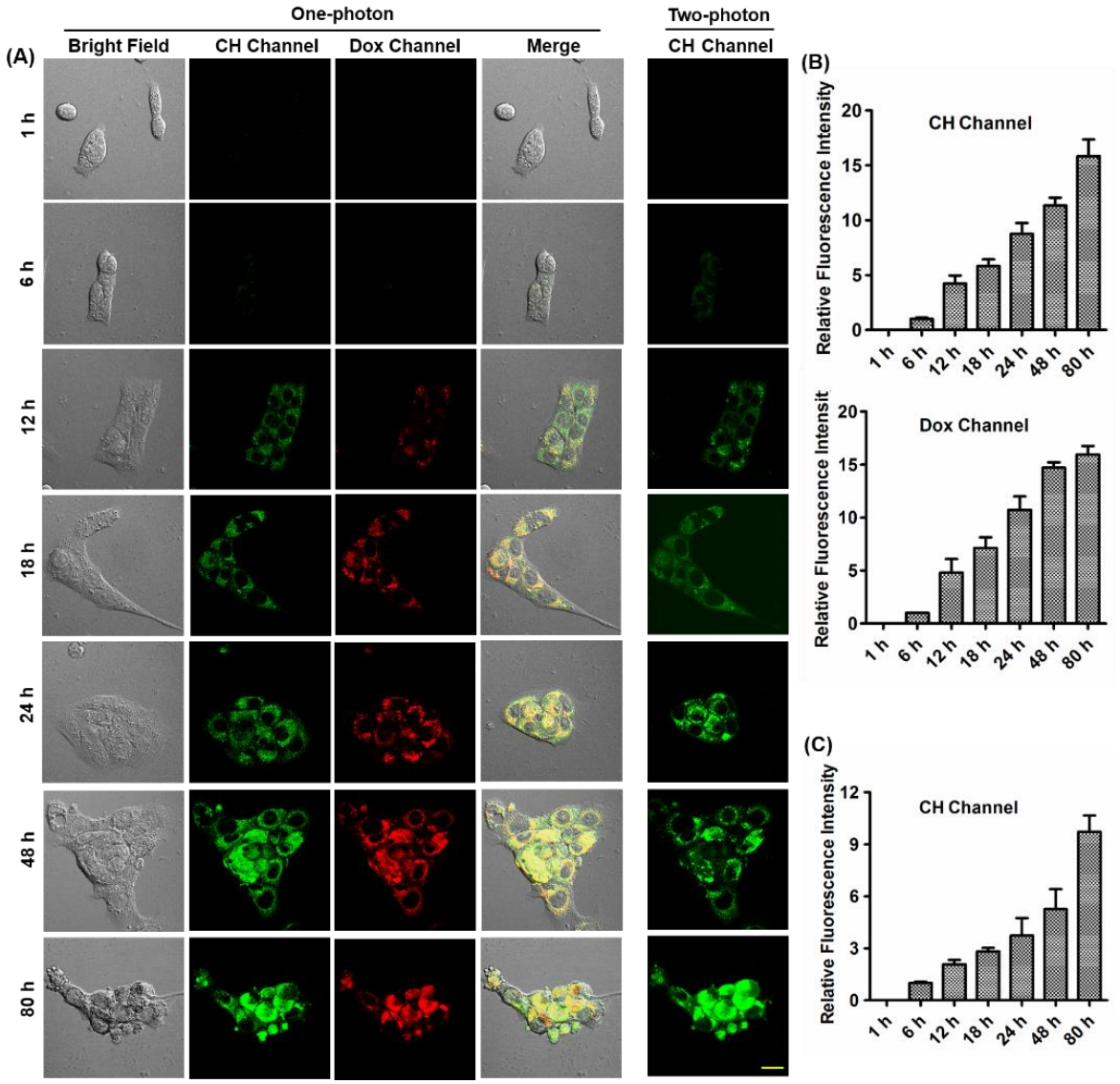


**Figure S5.** (A) Fluorescence images of 4T-1 cells treated with 5 μM **CDox** for different times in both one-photon (left) and two-photon (right) modes. One-photon mode: **CH** channel: $\lambda_{ex}$ = 405 nm, $\lambda_{em}$ = 425-475 nm, **Dox** channel: $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 570-620 nm; two-photon mode: **CH** channel: $\lambda_{ex}$ = 800 nm, $\lambda_{em}$ = 425-475 nm. Scale bar: 20 μm. (B) Quantified relative fluorescence intensities of **CH** and **Dox** in one-photon mode for different incubation times. (C) Quantified relative fluorescence intensities of **CH** in two-photon mode for different incubation times. Error bars represent standard deviation (±S.D.), n = 3.

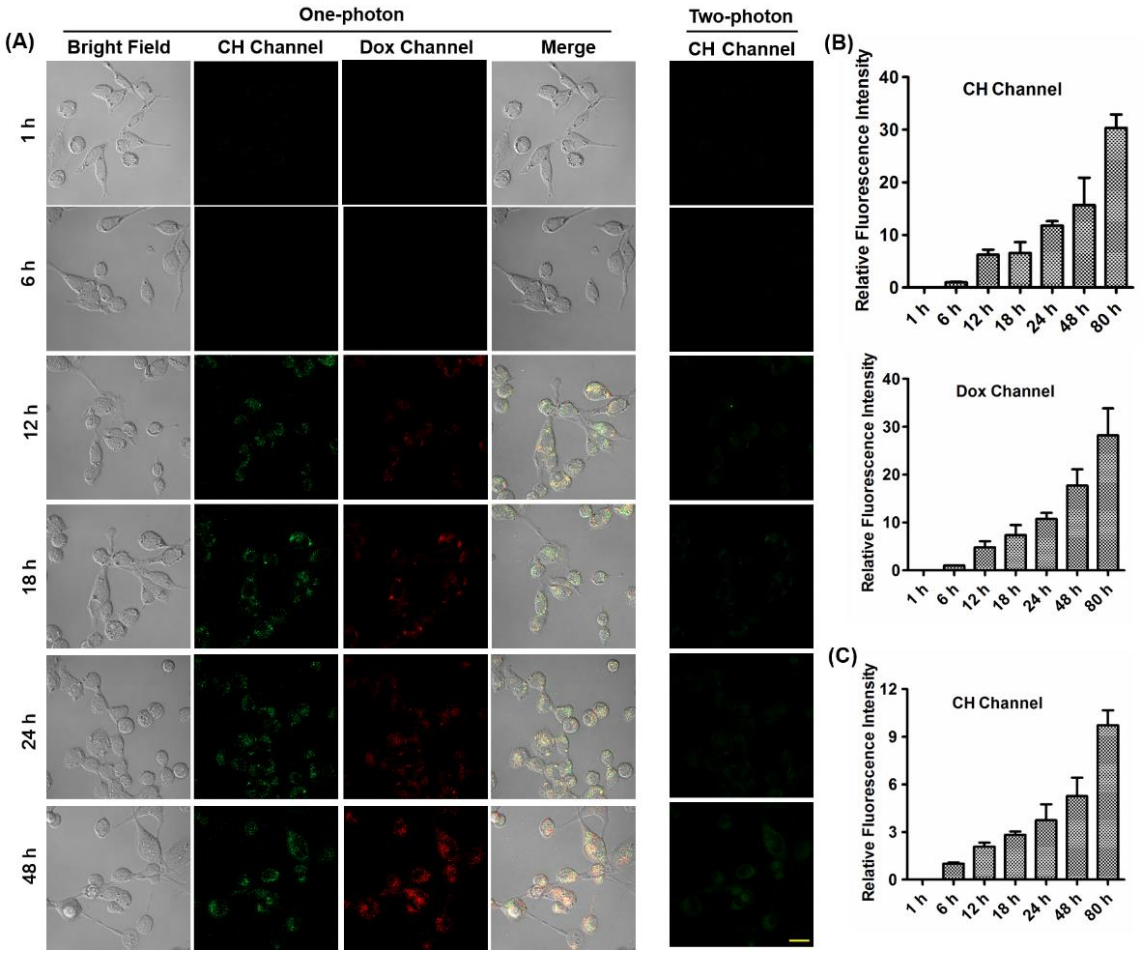


**Figure S6.** (A) Fluorescence images of HL-7702 cells treated with 5 μM **CDox** for different times in one-photon (left) and two-photon (right) modes. One-photon mode: **CH** channel: $\lambda_{ex}$ = 405 nm, $\lambda_{em}$ = 425-475 nm, **Dox** channel: $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 570-620 nm; two-photon mode: **CH** channel: $\lambda_{ex}$ = 800 nm, $\lambda_{em}$ = 425-475 nm. Scale bar: 20 μm. (B) Quantified relative fluorescence intensities of **CH** and **Dox** in one-photon mode for different incubation times. (C) Quantified relative fluorescence intensities of **CH** in two-photon mode for different incubation times. Error bars represent standard deviation (±S.D.), n = 3.

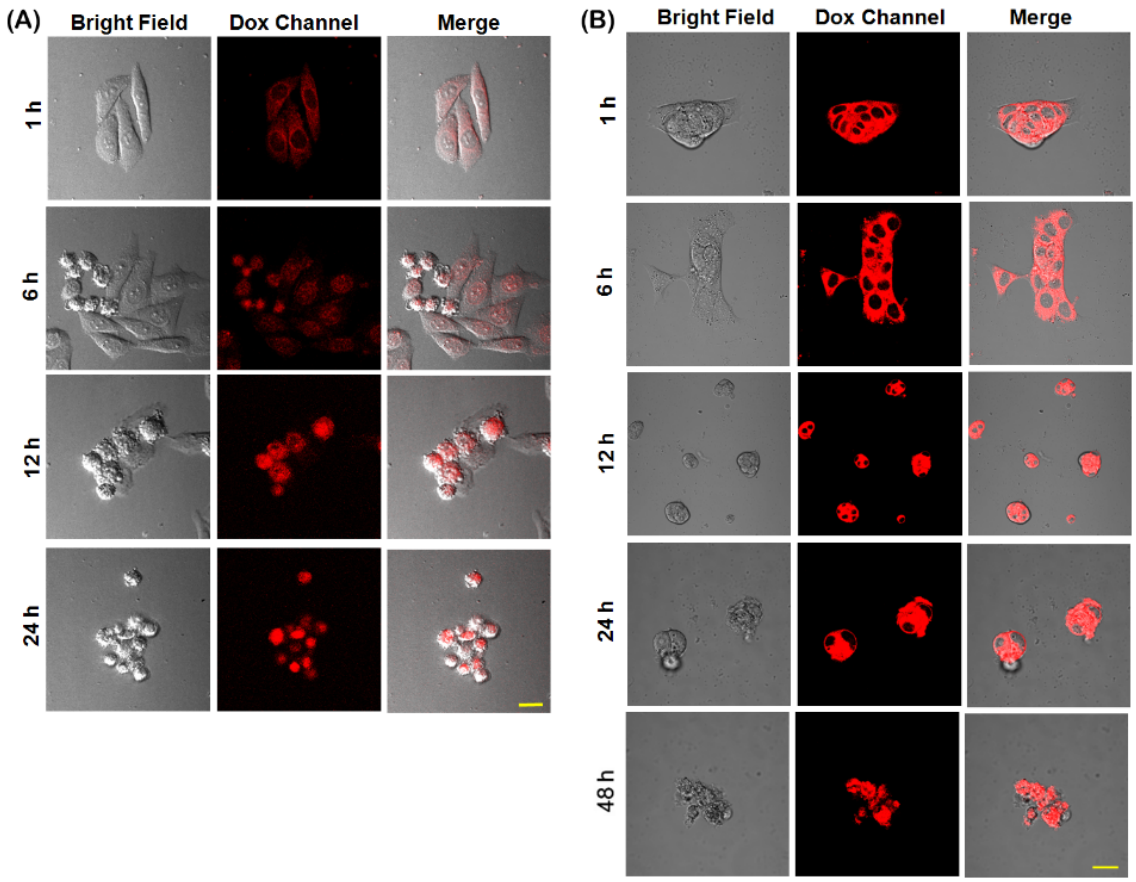


**Figure S7**. (A) Fluorescence images of the HepG2 cells incubated with **Dox** for different times. (B) Fluorescence images of the 4T-1 cells incubated with **Dox** for different times. $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 570-620 nm. Scale bar: 20 μm.

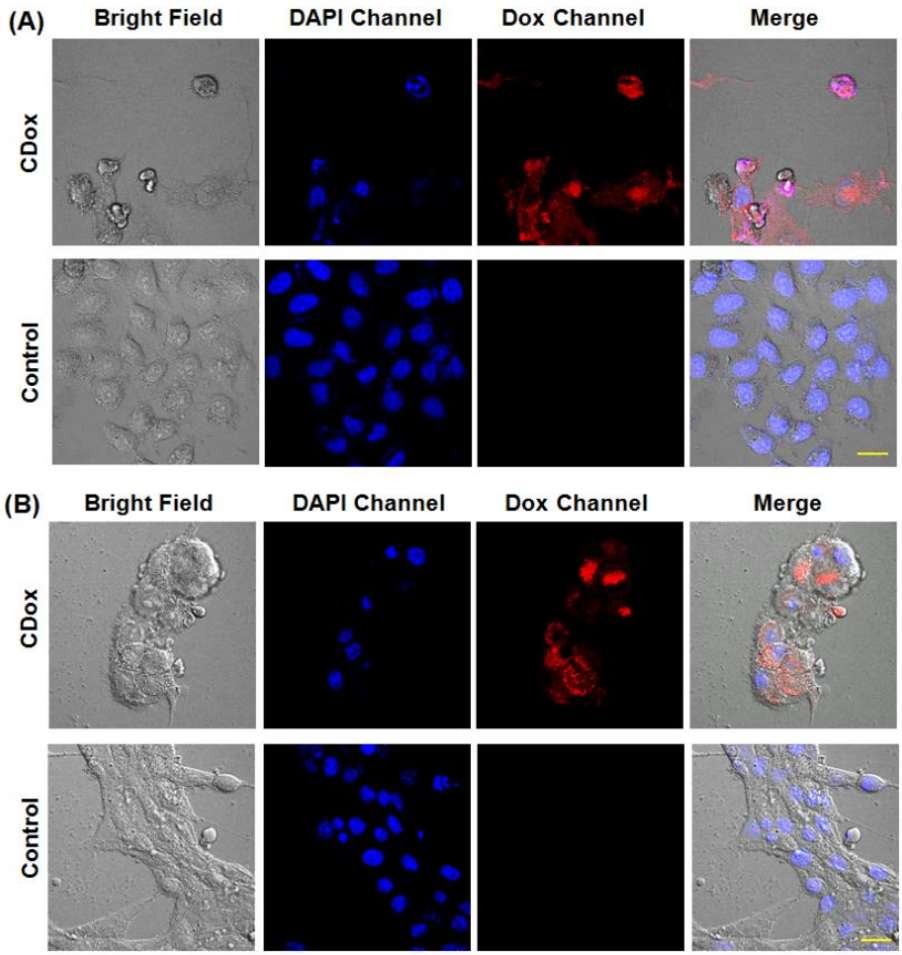


**Figure S8.** (A) Fluorescence images of the HepG2 cells incubated with or without **CDox** for 48 h. (B) Fluorescence images of the 4T-1 cells incubated with or without **CDox** for 80 h. Cells were fixed with 4% paraformaldehyde and incubated with DAPI (4 μg/mL). Scale bar: 20 μm

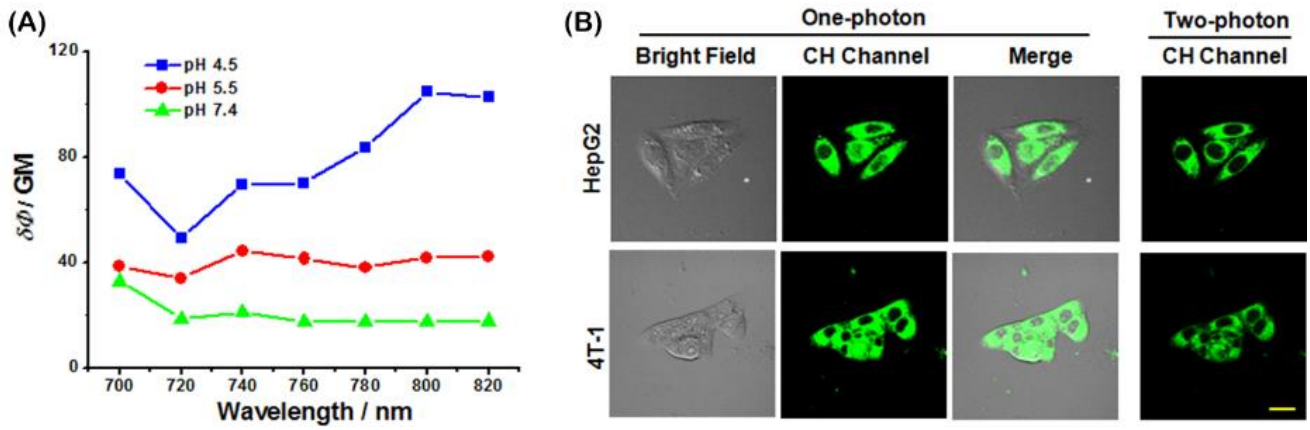


**Figure S9.** (A) Two-photon action ($\delta\Phi$) spectra of **CH** in B-R buffers (10% DMSO) at pH 4.5, 5.5 and 7.4, respectively. (B) One-photon and two-photon fluorescence images of the HepG2 cells and 4T-1 cells incubated with **CH** for 1 h.

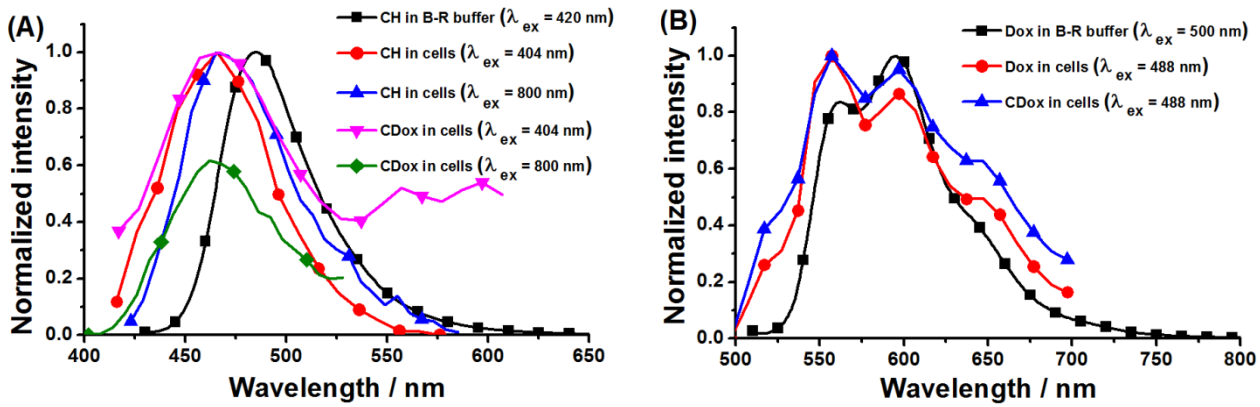


**Figure S10.** (A) Normalized fluorescence spectra of **CH** in B-R buffer (10% DMSO) and in HepG2 cells after an incubation of 1 h, and normalized fluorescence spectra **CDox** in HepG2 cells after an incubation of 48 h. (B) Normalized fluorescence spectra of **Dox** in B-R buffer (10% DMSO) and in HepG2 cells after an incubation of 1 h ($\lambda_{ex}$ = 488 nm), and normalized fluorescence spectra of **CDox** in HepG2 cells after an incubation of 48 h.

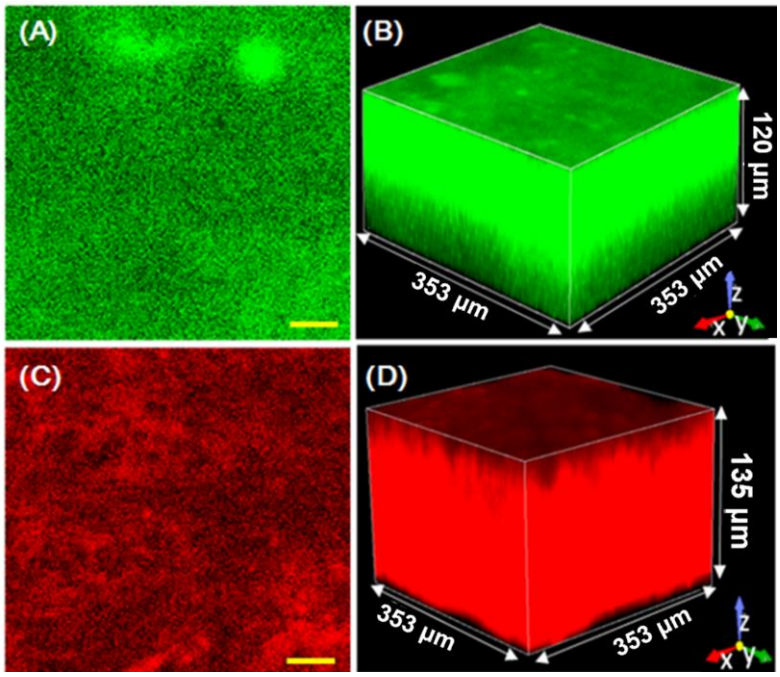


**Figure S11.** (A) Representative two-photon fluorescence images at a depth of 40 μm of the tumor tissues incubated with 10 μM **CH** for 2 h; $\lambda_{ex}$ = 800 nm, $\lambda_{em}$ = 425-475 nm, scale bar: 50 μm. (B) 3D imaging in two-photon mode. (C) Representative one-photon fluorescence images at a depth of 40 μm of the tumor tissues incubated with 10 μM **Dox** for 2h; $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 570-620 nm, scale bar: 50 μm. (D) 3D imaging in one-photon mode.

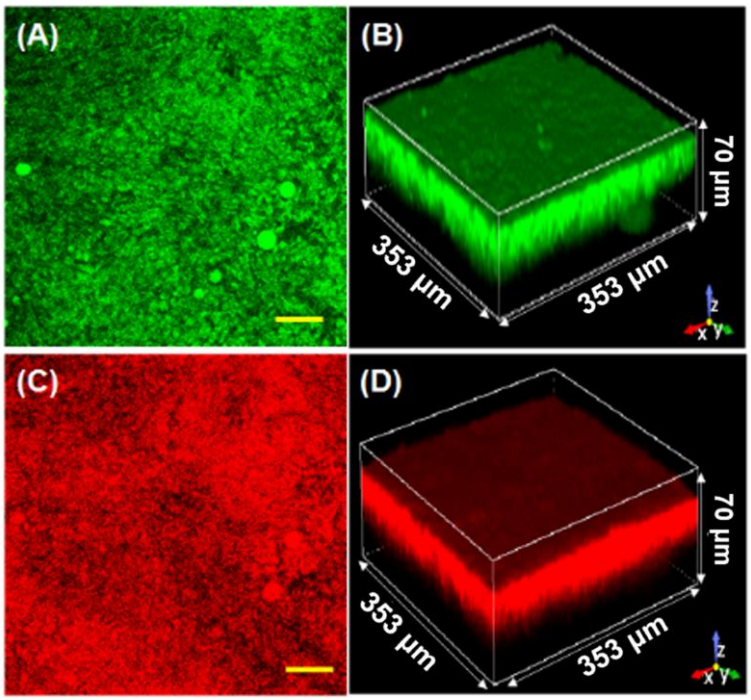


**Figure S12.** Images of living tumor tissue stained with 10 μM **CDox** for 0.5 h. (A) Representative two-photon images at a depth of 40 μm; $\lambda_{ex}$ = 800 nm, $\lambda_{em}$ = 425-475 nm, scale bar: 50 μm. (B) 3D imaging in two-photon mode. (C) Representative one-photon images at a depth of 40 μm, $\lambda_{ex}$ = 488 nm; $\lambda_{em}$ = 570-620 nm, scale bar: 50 μm. (D) 3D imaging in one-photon mode.

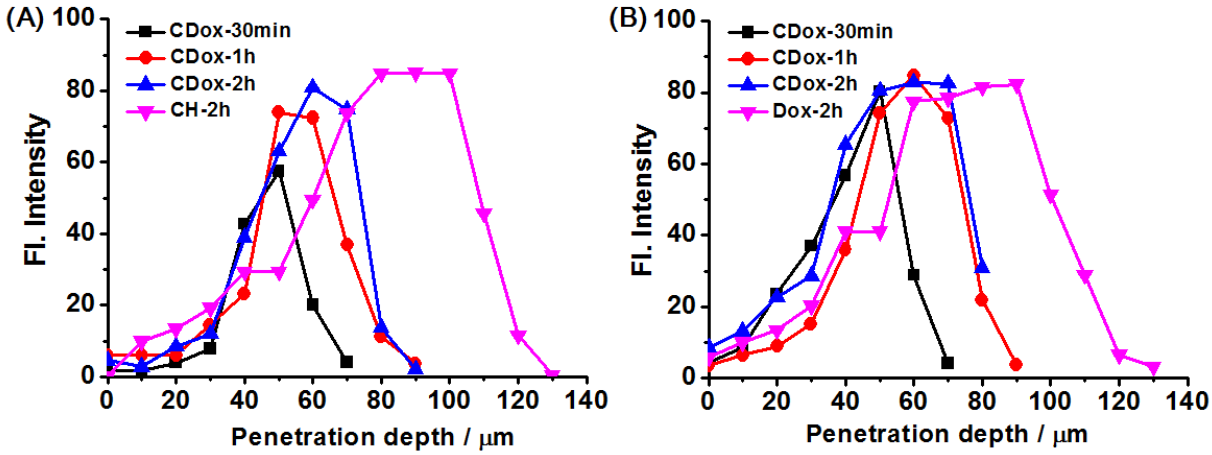


**Figure S13**. Time-dependent quantified fluorescence intensities of **CDox**, **CH** and **Dox** in different penetration depth. (A) Quantified fluorescence intensity of **CDox** and **CH** in two-photon channel ($\lambda_{ex}$ = 800 nm, $\lambda_{em}$ = 425-475 nm). (B) Quantified fluorescence intensity of **CDox** and **Dox** in one-photon channel ($\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 570-620 nm). Mean fluorescence intensities were measured by Image J Software.

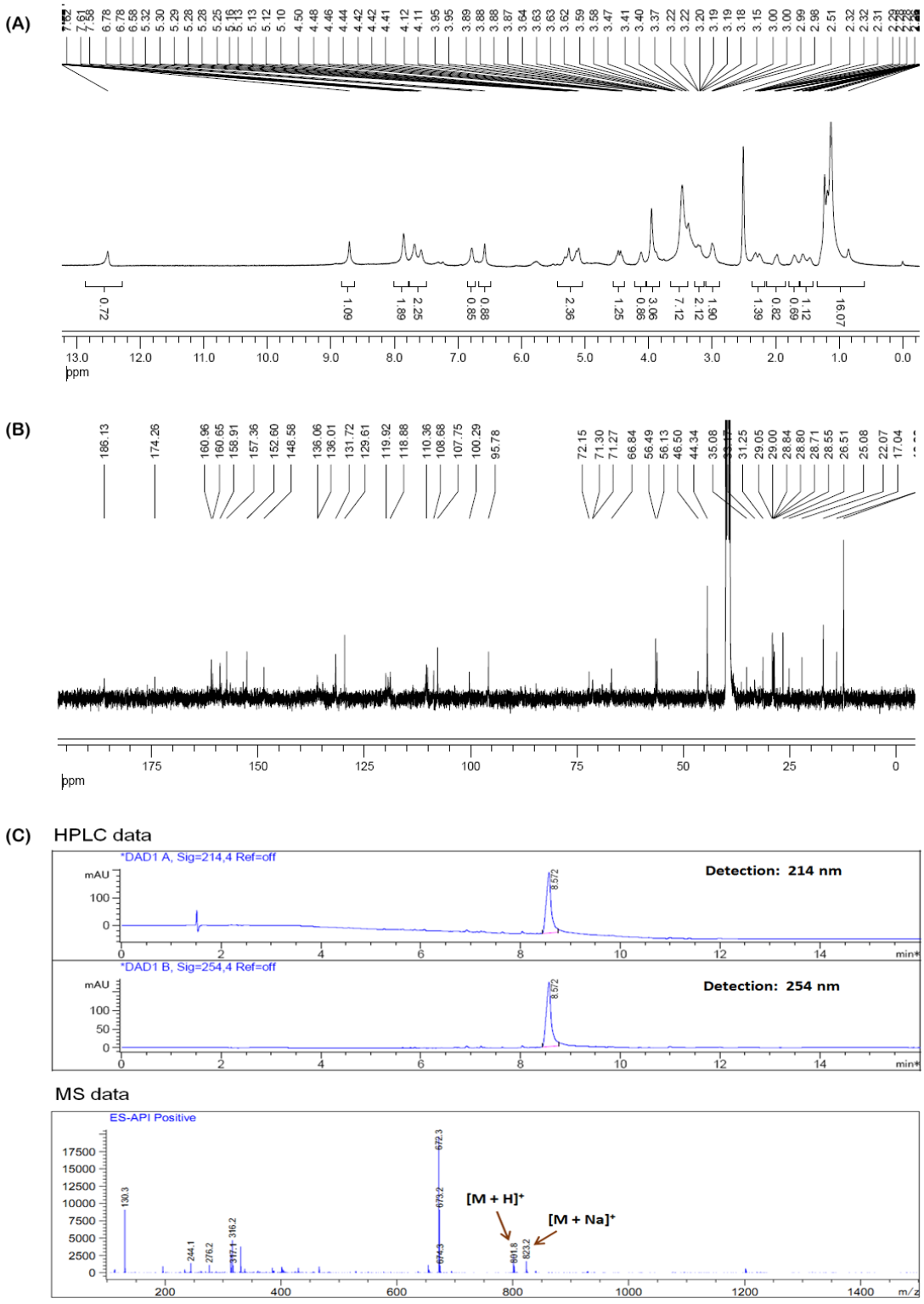


**Figure S14.** $^{1}$HNMR (A), $^{13}$C NMR (B) and LC-MS (C) data of **CDox**.