Experimental details

General

The solvents used are of ACS grade or HPLC grade. The ligand mbc94 was synthesized using the previously reported procedure.[33] The NIR dye, IR700DX-NHS ester, was purchased from LI-COR Bioscience (product number 929-70011). Flash column chromatography was performed using the TELEDYNE ISCO (combiflash RF) purification system with silica gel (standard grade, 60A, Sorbtech). ^{1}H NMR spectra were recorded on the Brucker Avance III 400 MHz. Mass spectra were recorded on a Waters LCT Premier mass spectrometer. UV/Vis spectra were recorded on a Cary 100 Bio UV-Vis spectrophotometer, and fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer.

Characterization of IR700DX-mbc94

^{1}H NMR (400 MHz, *d4*-MeOD, 25 ºC, TMS): δ = 9.74-9.77 (m, 5 H), 9.61-9.63 (m, 1 H), 9.43 (d, *J* = 7.2 Hz, 1 H), 8.55 (s, 1 H), 8.48-8.50 (m, 5 H), 8.45 (d, *J* = 8 Hz, 1 H), 8.06 (s, *J* = 8 Hz, 1 H), 7.39 (d, *J* = 8 Hz, 2 H), 7.30-7.34 (m, 2 H), 7.19 (dd, *J* = 8 & 1.6 Hz, 1 H), 7.05 (d, *J* = 8 Hz, 2 H), 6.86 (s, 1 H), 5.48 (s, 2 H), 4.60 (br.s, 12 H), 4.00 (s, 1 H), 3.77 (d, *J* = 2 Hz, 1 H), 3.13-3.17 (m, 4 H), 2.73-2.79 (m, 26 H), 2.34 (s, 3 H), 2.19 (t, *J* = 7.2 Hz, 2 H), 2.00-2.04 (m, 2H), 1.69-1.80 (m, 14 H), 1.49-1.65 (m, 9 H), 1.29-1.39 (m, 6 H), 1.15 (s, 3 H), 1.08 (s, 3 H), 0.86 (s, 3 H), -0.96 (br.s, 4 H), -2.11- -2.09 (m, 4 H), -2.77 (s, 12 H). MS (ESI): calcd. for $C_{105}H_{143}ClN_{16}O_{25}S_6Si_3$ *m/z* 2339.78, found *m/z* 2339.95.

Cell Culture

In vitro studies were performed using a mouse malignant astrocytoma cell line transfected with CB_2R, CB_2-mid DBT (delay brain tumor), which expresses CB_2R at endogenous levels, as well as a wild type (WT) DBT cell line without CB_2R expression. CB_2-mid DBT and WT-DBT cells were cultured in DMEM containing 10% fetal bovine serum, 4 mM glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin. The human CB_2-expressing CHO-K1/CB2 cells were cultured according to the previously published literature (Gertsch, et al., 2008).

In Vivo Phototherapy

The animal experiments are in accordance with the guidelines for the Care and Use of Laboratory Animals of the Medical Research Council of University of Pittsburgh. CB_2-mid DBT cells (1×10^6) were subcutaneously implanted into the right flank of 4−5 week old female nu/nu mice. Mice with tumor volume reaching approximately 40-50 mm^3 were selected for study. Selected mice were randomized into non-treatment and phototherapy treatment groups (n=3 for each group). For phototherapy treatment, tumor bearing mice were intravenously injected with 10 nmole IR700DX-mbc94 via tail vein. After 6 h post-injection at which IR700DX-mbc94 reached a peak tumor accumulation (data not shown), mice were anesthetized with 2.5% isoflurane and tumor area was irradiated under NIR light (90 J/cm^2) using the same light source as in vitro phototherapy study. In vivo phototherapy study was conducted every 5 days. The tumor volume was measured daily using a caliper and tumor volume was calculated by length × width^2/2. Photographic images were captured by a charge-coupled device camera-based bioluminescence imaging system (IVIS Lumina XR). When tumor diameters reached 15 mm, mice were euthanized with carbon dioxide gas.

Data Processing and Statistics

All of the data are given as the mean ± standard deviation (SD) of n independent measurements. Statistical analysis was performed using a two-tailed unpaired Student's t test (IBM SPSS Statistics version 21), with p values <0.05 considered statistically significant.

Supplementary Figures

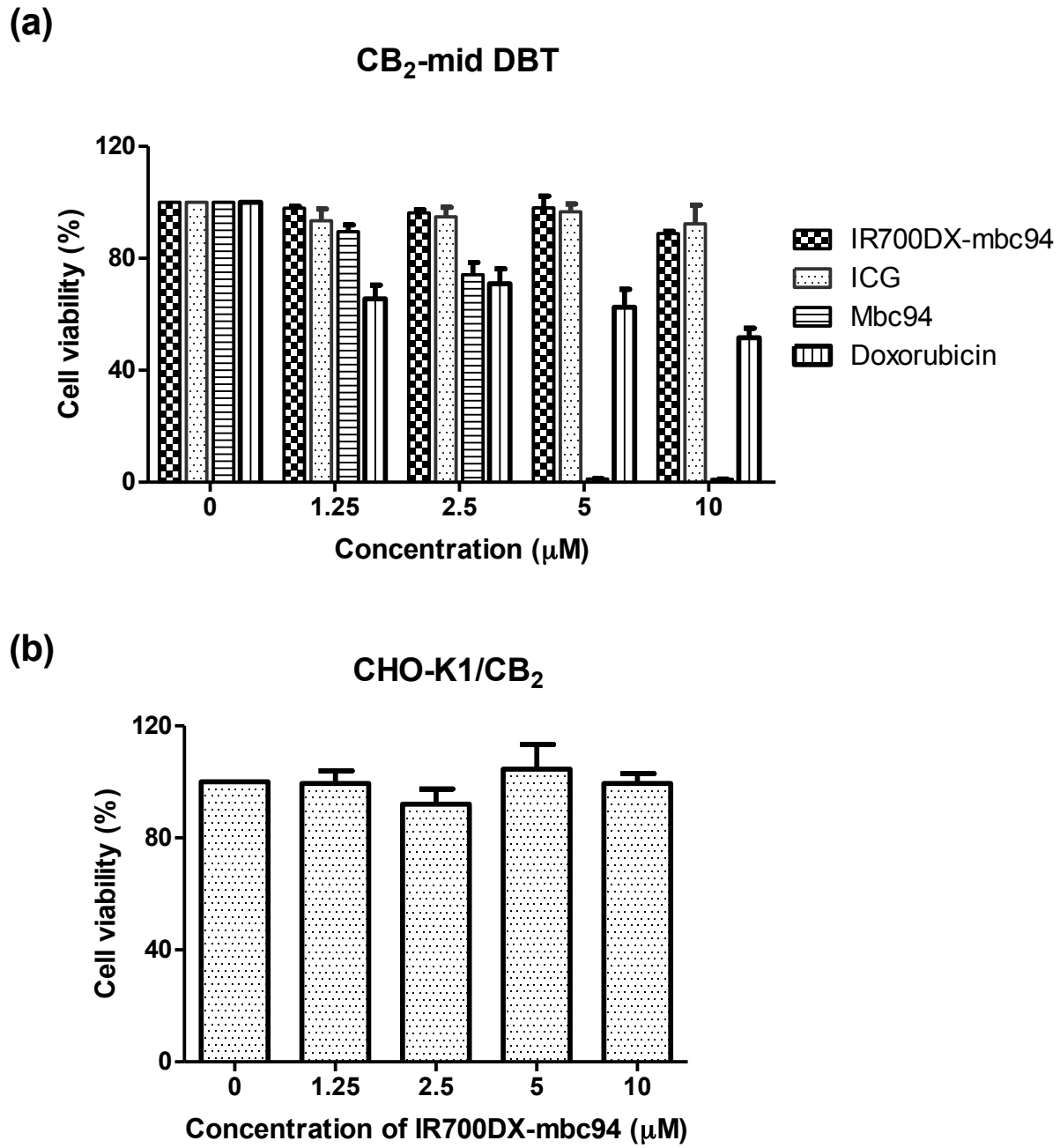

Figure S1. Cytotoxicity of IR700DX-mbc94 (relevant to Experimental Procedures).

(a) To evaluate the cytotoxicity of IR700DX-mbc94 without NIR light irradiation, CB_2-mid DBT cells were incubated with 0, 1.25, 2.5, 5, 10 μM of IR700DX-mbc94 or Mbc94 alone for 24 h. ICG with the same concentration of IR700DX-mbc94 was used as the negative control. Doxorubicin was used as the positive control. (b) To test the toxicity of the IR700DX-mbc94 in cells that express the CB_2 receptor but are not tumoral, CHO-K1/CB2 cells were treated with indicated concentration of IR700DX-mbc94 (0, 1.25, 2.5, 5, 10 μM) for 24 h. Cell viability was measured using the CellTilter-Glo Luminescent Cell Viability assay. Each data point represents the mean ± SD based on triplicate samples.

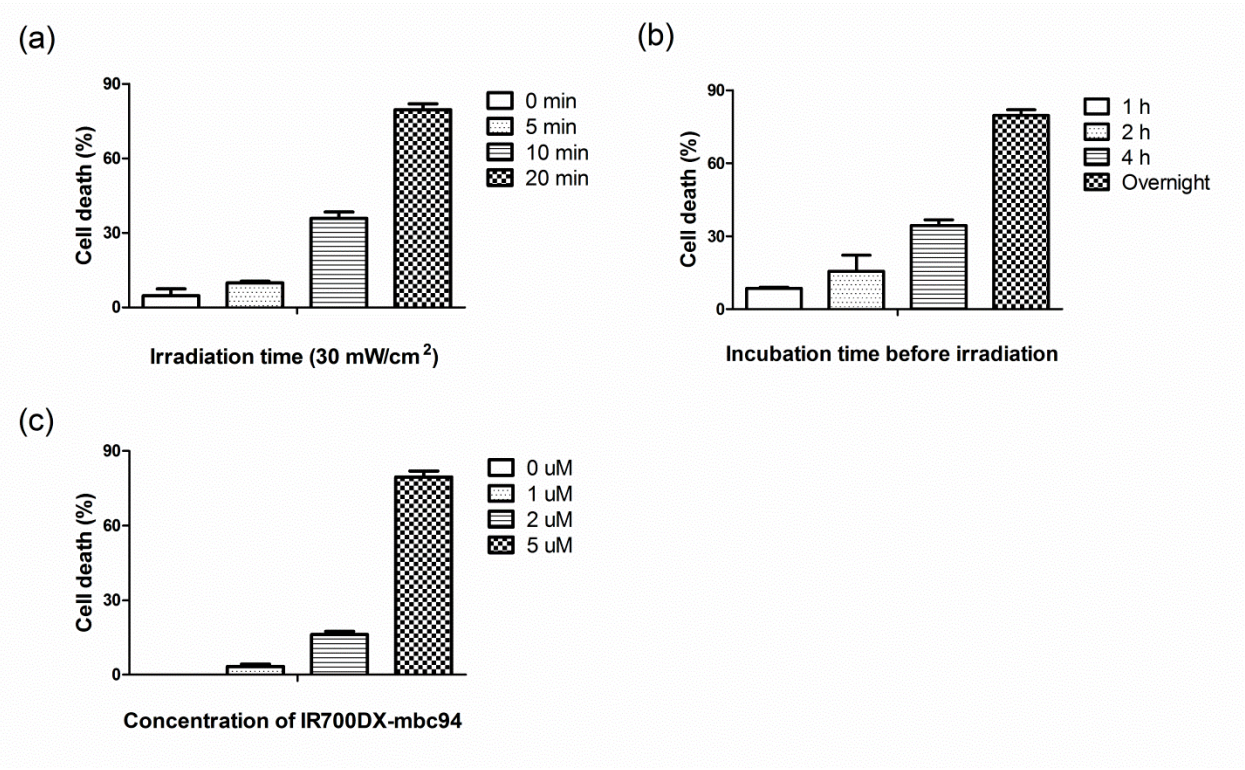

Figure S2. Effect of light irradiation dose, incubation time and concentration of IR700DX-mbc94 on phototherapy (relevant to Figure 3).

CB_2-mid DBT cells were treated with (a) 5 μM of IR700DX-mbc94 with 30 mW/cm^2 NIR light exposure for 0 min, 5 min, 10 min, and 20 min; (b) 5 μM of IR700DX-mbc94 with an incubation time of 1 h, 2 h, 4 h or overnight before NIR light irradiation (30 mW/cm^2, 20 min); (c) 0, 1, 2, or 5 μM of IR700DX-mbc94 overnight, and irradiated with NIR light (30 mW/cm^2, 20 min). Each data point represents the mean ± SD based on triplicate samples.

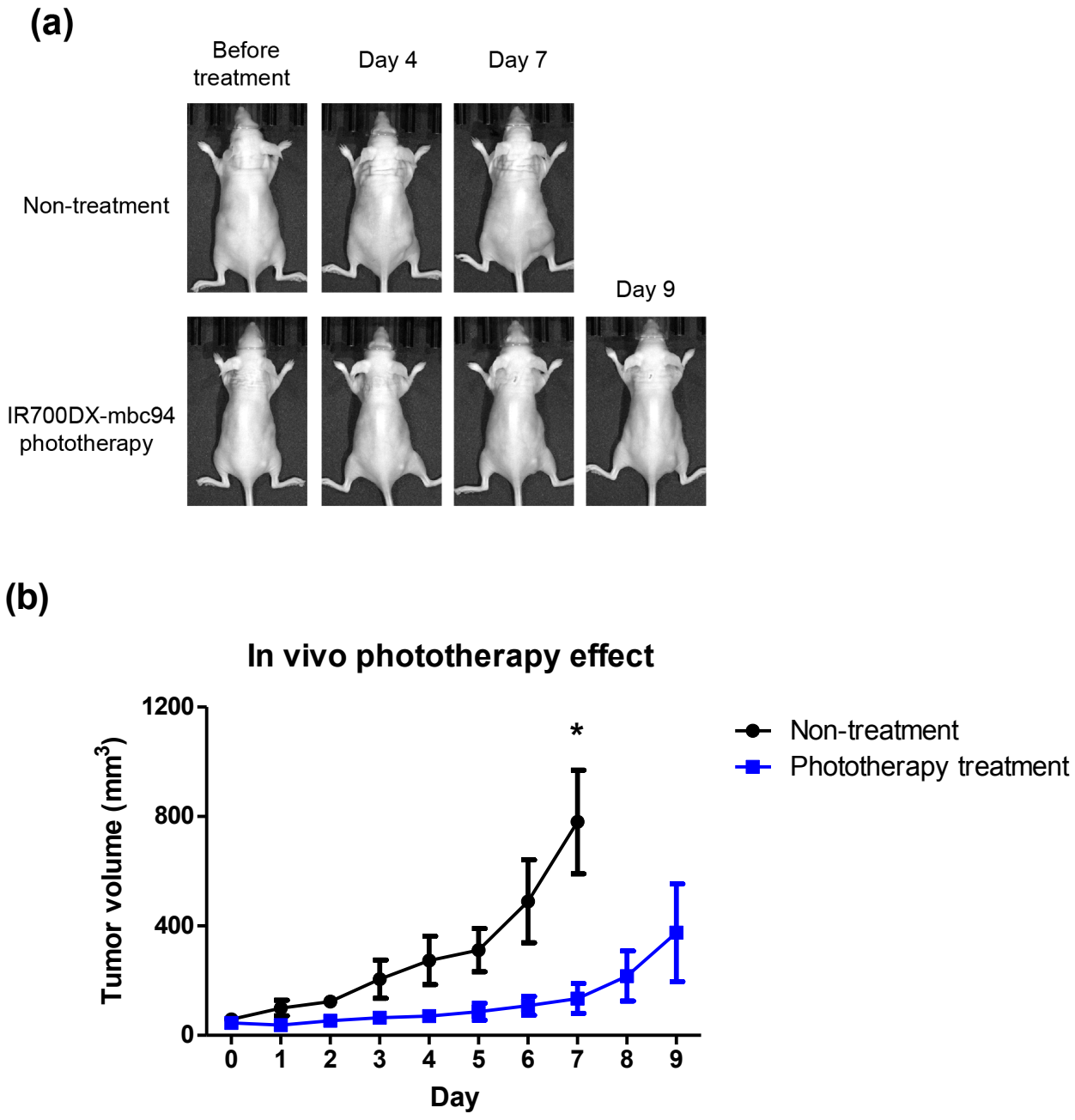

Figure S3. In vivo phototherapy study (relevant to Figure 3).

CB_2-mid DBT subcutaneous tumor bearing mice were intravenously injected with 10 nmole IR700DX-mbc94 via tail vein. After 6 h post-injection mice were anesthetized with 2.5% isoflurane and tumor area was irradiated under NIR light (90 J/cm^2). In vivo phototherapy was conducted at day 0 and day 5. The tumor volume was measured daily. (a) Photographic images were captured by a charge-coupled device camera-based bioluminescence imaging system. (b) Quantitative comparison of tumor growth between phototherapy treatment and non-treatment group. Each data point represents the mean ± SD based on three animals (* p<0.05).

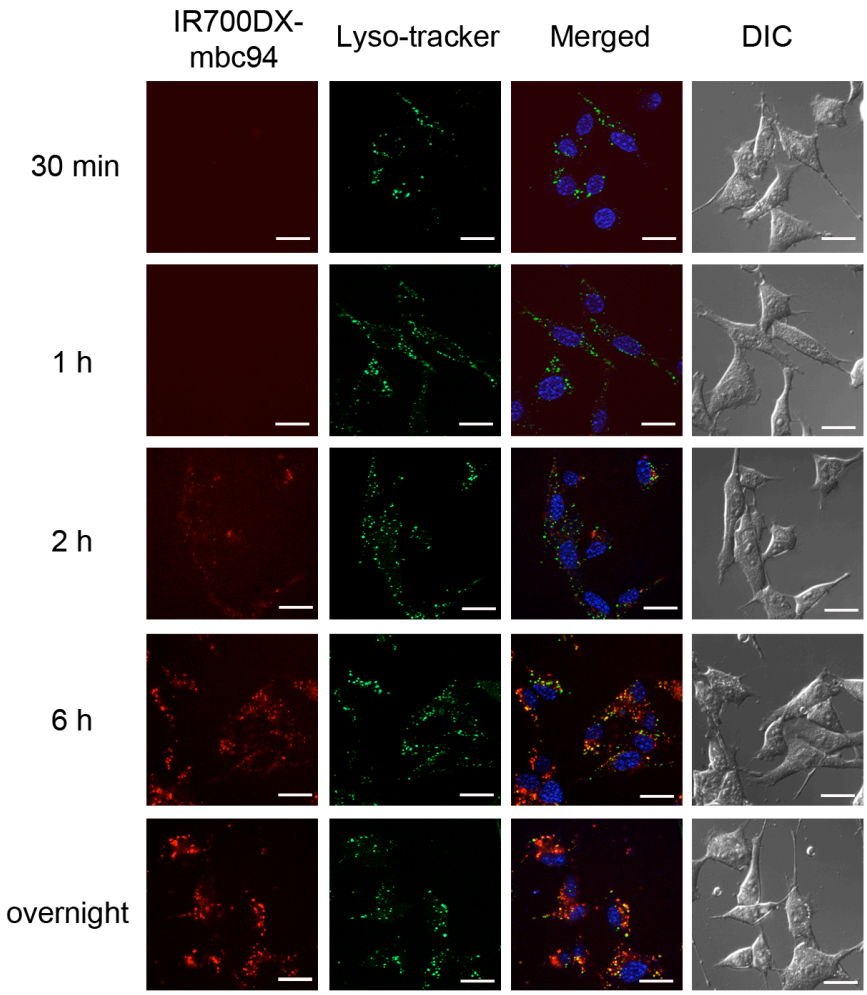

Figure S4. Localization of IR700DX-mbc94 in CB_2-mid DBT cells (relevant to Figure 4 and Experimental Procedures).

CB_2-mid DBT cells were treated with 5 μM of IR700DX-mbc94 at 37 °C for 30 min, 1 h, 2 h, 6 h and overnight. Co-localization of IR700DX-mbc94 with lysosome (LysoTracker) and nuclear (DAPI) was imaged under Zeiss Axio Observer fluorescent microscopy. Certain co-localization (orange color) of LysoTracker and IR700DX-mbc94 fluorescence after 2 h of incubation was observed, indicating that part of IR700DX-mbc94 bound to surface CB_2R and internalized afterwards. The majority of IR700DX-mbc94 molecules bound to intracellularly expressed CB_2R.

Reference

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J.Z., Xie, X.Q., Altmann, K.H., Karsak, M., and Zimmer, A. (2008). Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci U S A 105, 9099-9104.