Supporting Information

Synthesis and Characterization of a Cell-permeable Near-Infrared Fluorescent Deoxyglucose Analogue for Cancer Cell Imaging

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1. Experimental procedures and characterization data.

All the chemicals and solvents were purchased from Sigma Aldrich, Alfa Aesar or Acros, and used without further purification. **IRDye 800CW-OSu** was purchased from LI-COR Biosciences. Analytical characterization was performed on a HPLC-MS (Agilent-1200 series) with a DAD detector. Analytical method, unless indicated: eluents: A: H_2O (0.1% HCOOH), B: ACN (0.1% HCOOH), gradient from 5 to 95%B in 6 min; C_{18}(2) Luna column (4.6 x 50mm^2, 5μm particle size). High resolution mass spectrometry (HRMS) data was recorded on a Micromass VG 7035 (Mass Spectrometry Laboratory at National University of Singapore (NUS)). ^{1}H-NMR spectra were recorded on Bruker Avance 500 NMR spectrometer, and chemical shifts are expressed in parts per million (ppm). Spectroscopic data were measured on a SpectraMax M2 spectrophotometer (Molecular Devices), and the data analysis was performed using GraphPad Prism 5.0. Fluorescence microscopy experiments were performed in a Nikon Ti microscope attached to a CoolLED (740 nm) excitation source, and images were processed using the software NIS-Elements 3.10. Cell viability assays were performed using a CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS) (Promega) following the manufacturer's instructions.

Synthesis of CyNE 2-DG and IRDye 800CW 2-DG.

1 (prepared as reported in the reference 12 of the manuscript, 50 mg, 0.07 mmol) and HATU (25 mg, 0.07 mmol) were dissolved in 5 mL of anhydrous DMF under N_2 atmosphere and stirred at r.t. for 10 min. 2-D-deoxyglucosamine•HCl (15 mg, 0.07 mmol) was treated with DIEA (14 μL, 0.10 mmol) and added to the reaction mixture. The reaction was stirred for another 2 h at r.t., then the solvent was evaporated under reduced pressure and the resulting green solid was purified by normal-phase column chromatography (elution with DCM-MeOH (9:1)) to obtain **CyNE 2-DG** as a green solid (41 mg, yield 68%).

tR: 6.15 min; ESI (HRMS) calc. for $C_{56}H_{80}N_5O_7^+$: 934.6052, found: 934.6070.

Spectral properties (PBS pH 7.3, 1% DMSO): ε (790 nm) = 106,000 M^{-1} cm^{-1}, quantum yield = 0.03.

Indocyanine Green used as standard for quantum yield determination (Soper *et al.*, *J. Am. Chem. Soc.*, 1994, **116**, 3744.)

IRDye 800CW 2-DG was prepared as reported in the reference 10 of the manuscript.

tR: 7.94 min (RP-HPLC gradient from 5 to 100%B in 23 min)

ESI (HRMS) calc. for $C_{52}H_{63}N_3O_{19}S_4^{-3}$: 1161.3101, found ($z$=2): 580.6488.

NMR characterization of 2-D-deoxyglucosamine•HCl and CyNE 2-DG

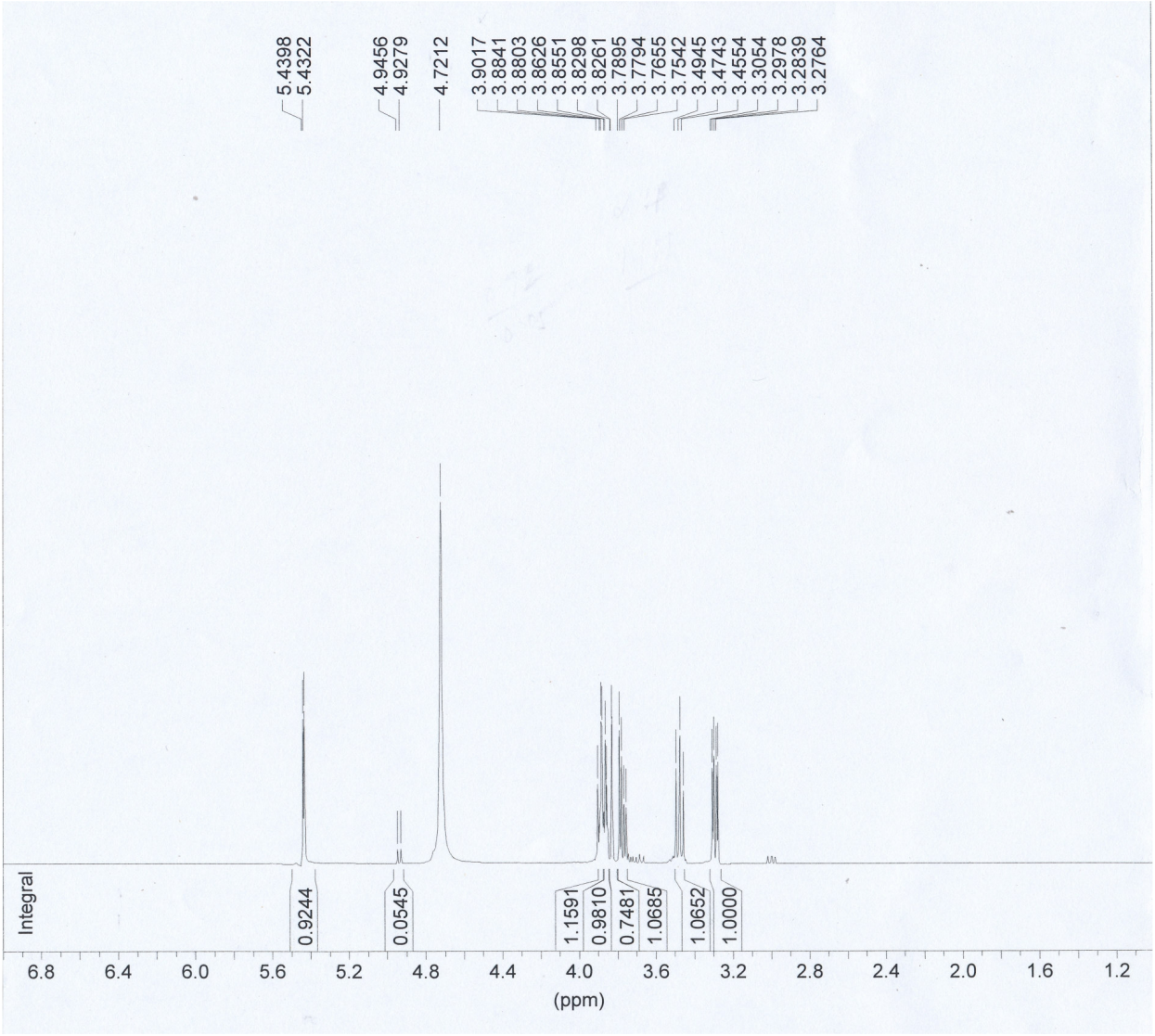

Figure S1. ^{1}H-NMR spectrum of 2-D-deoxyglucosamine•HCl in D_2O: 3.27 (dd, 1H, J_1 = 11 Hz, J_2 = 3.5 Hz), 3.47(t, J = 9.5 Hz), 3.75-3.78 (m, 1H), 3.82-3.90 (m, 3H), 4.94 (d, 0.05H$_\beta$, J = 8.8 Hz), 5.43 (d, 0.93H$_\alpha$, J = 3.5 Hz).

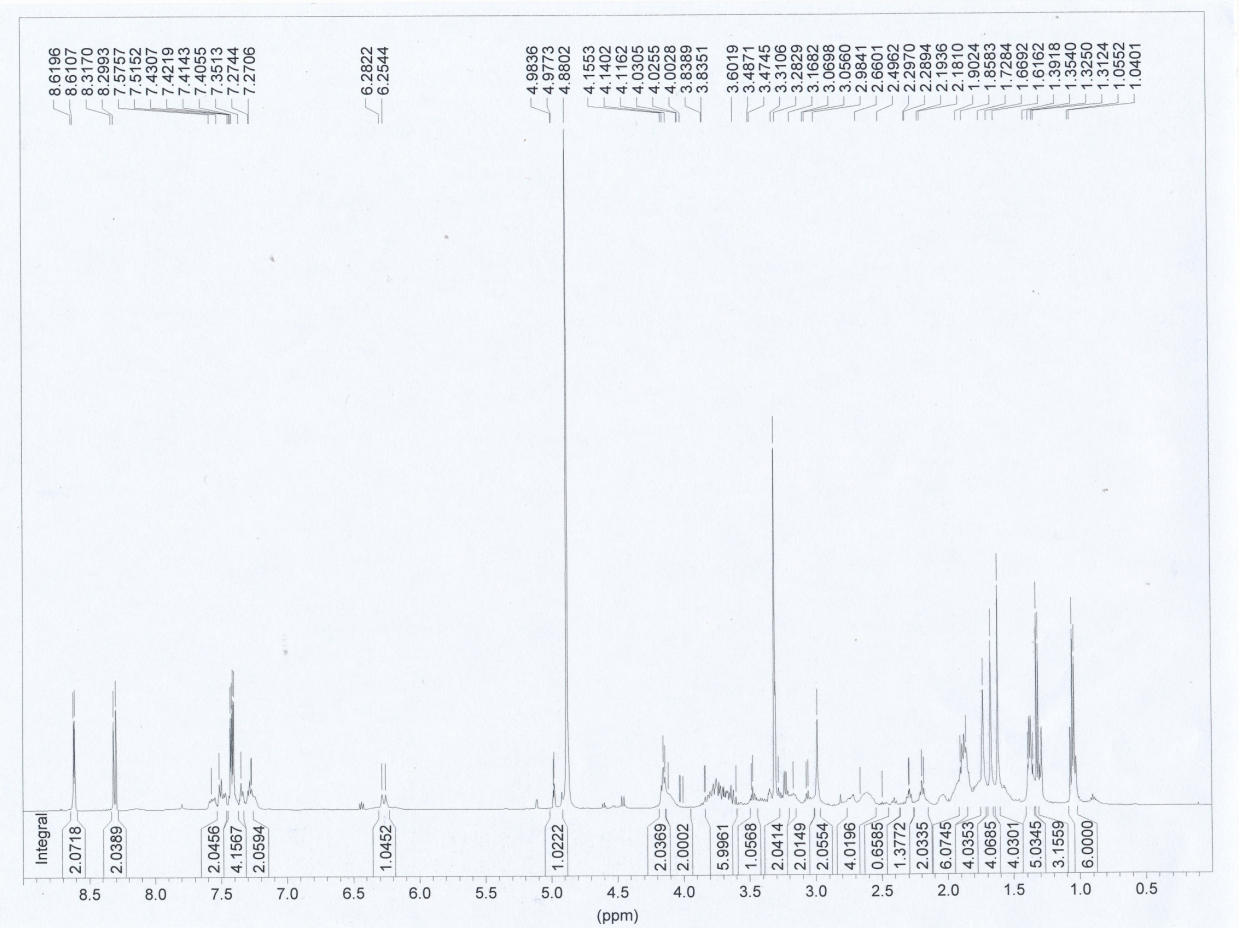

Figure S2. ^{1}H-NMR spectrum of **CyNE 2-DG** in MeOD: 1.04 (t, 6H, *J* = 7.5Hz), 1.32 (d, 3H, *J* = 6.6 Hz), 1.35-1.39 (m, 5H), 1.61 (s, 4H), 1.66 (s, 4H), 1.72 (s, 4H), 1.83-1.90 (m, 6H), 2.18 (t, 2H, *J* = 8.0 Hz), 2.28-2.39 (m, 2H), 2.52-2.62 (m, 4H), 2.98 (t, 2H, *J* = 5.4 Hz), 3.05 (t, 2H, *J* = 5.4 Hz), 3.06-3.26 (m, 4H), 3.47 (t, 4H), 3.60-3.83 (m, 6H), 4.06 (m, 2H), 4.15 (t, 2H, *J* = 7.5 Hz), 4.97 (d, 1H, *J* = 3.2 Hz), 6.25 (d, 1H, *J* = 14.1 Hz), 7.24-7.58 (m, aromatic 8H), 8.29 (d, 2H, *J* = 10.5 Hz), 8.61 (d, 2H, *J* = 3.5 Hz).

Fluorescence microsopy analysis

MCF7 and MDA-MB-231 cells were purchased from American Type Culture Collection (ATCC) and maintained in DMEM containing 10% fetal bovine serum (FBS). Serum-starved cultures of different cell lines were incubated at 37 ^{o}C for 20 min with 20 μM dyes (**CyNE 2-DG** or **IRDye 800CW 2-DG**) in low-glucose DMEM media, washed with PBS (×1) and treated with Hoechst for nuclear staining (37 ^{o}C, 10 min). For competition experiments with D-glucose and L-glucose, cells were incubated at 37 ^{o}C for 3 h with low-glucose DMEM containing 0, 10 or 50 mM glucose. **CyNE 2-DG** was then added incubated at 37 ^{o}C for 20 min, washed with PBS (×1) and treated with Hoechst for nuclear staining (37

^{o}C, 10 min). Cell images were taken in a Nikon Ti microscope at 10× magnification with 360±20/460±25 and 750/800 filter cubes for Hoechst and NIR fluorescence images respectively.

2. Optimization of concentration for CyNE 2-DG: cell uptake and toxicity assays.

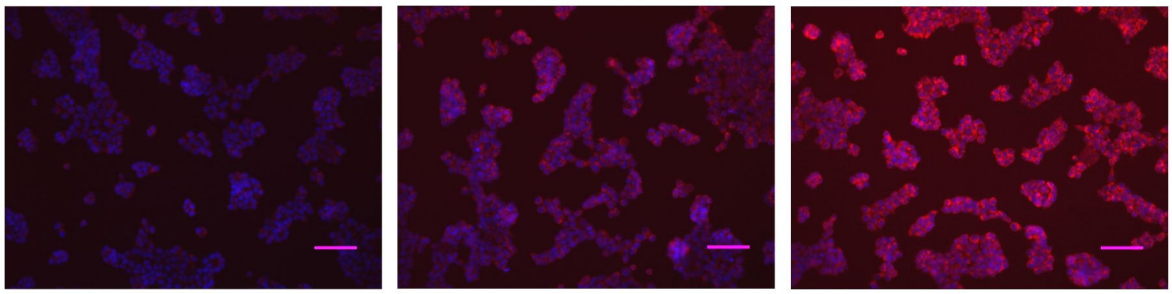

Figure S3. Fluorescence images of MCF7 cells upon incubation with **CyNE 2-DG** (*left*: 10 μM, *center*: 20 μM, *right*: 50 μM) at 37 °C for 20 min followed by nuclear staining with Hoechst. Fluorescence images were taken under the same acquisition conditions. NIR fluorescence is shown in red color and Hoechst staining in blue color. Scale bar: 100 μm.

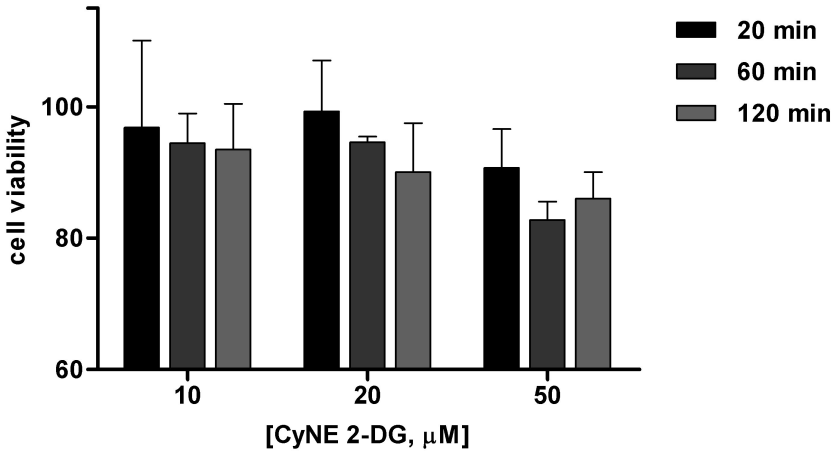

Figure S4. Cell viability of **CyNE 2-DG** at different concentrations and incubation times in MCF7 cells. Values are referred to untreated cells (100% viability) and plotted as average from 3 replicates with error bars as standard deviations.

3. Competition experiments of CyNE 2-DG with L-glucose.

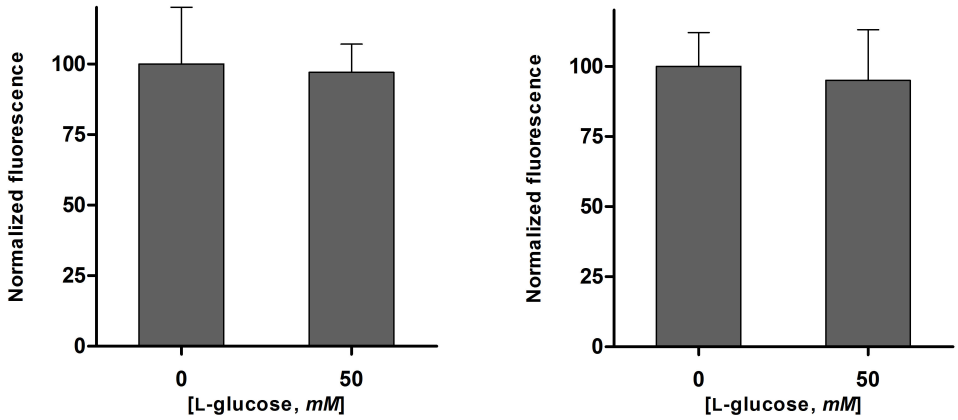

Figure S5. **CyNE 2-DG** uptake after competition with L-glucose in MCF7 (*left*) and MDA-MB-231 (*right*) cells. Fluorescence intensity values from 5 to 10 different regions were averaged and referred to the fluorescence intensity measured in cells with no glucose competition. Error bars correspond to standard deviations.

4. Fluorescence images of MCF7 and MDA-MB-231 cells upon incubation with CyNE 2-DG or 1.

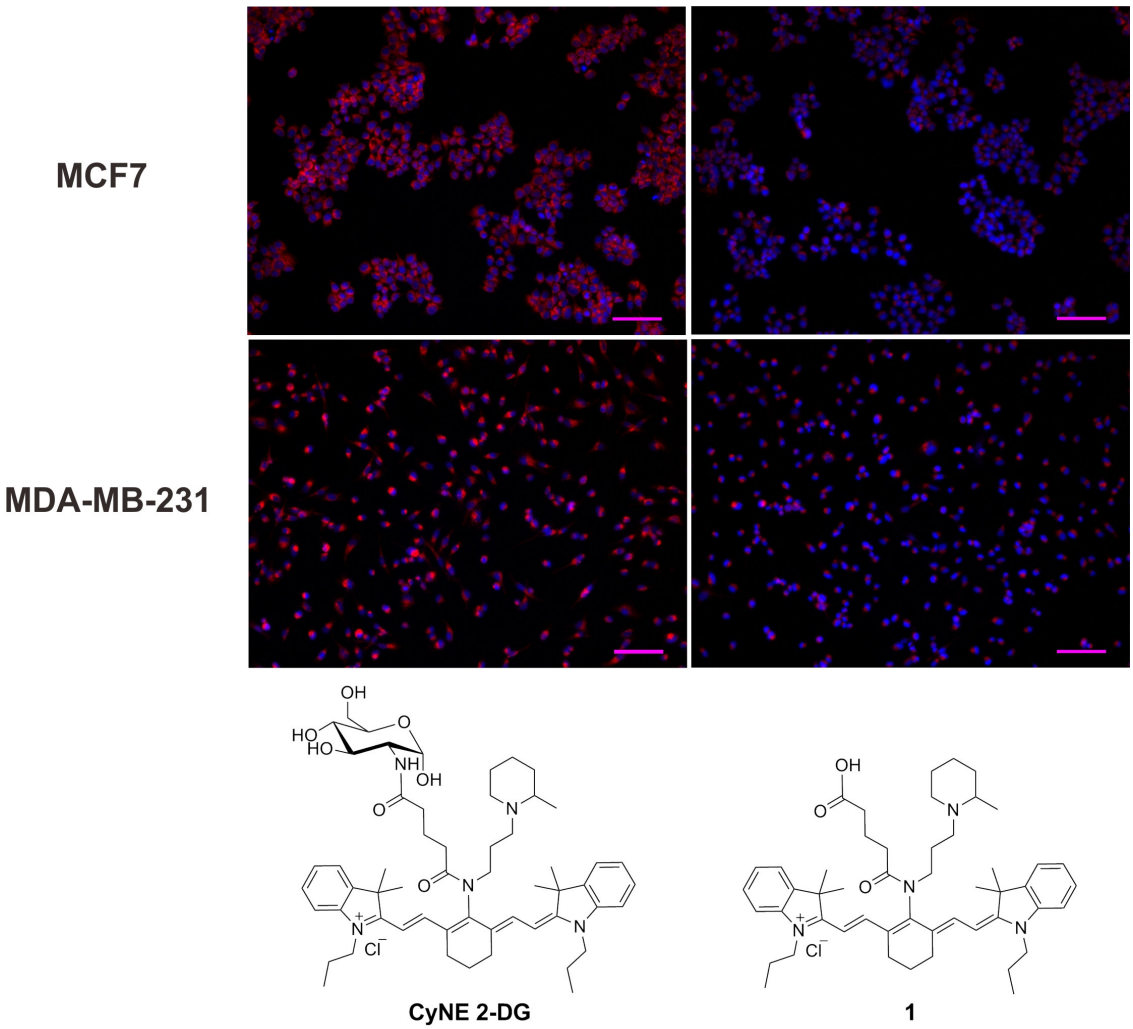

Figure S6. Fluorescence images of MCF7 and MDA-MB-231 cells upon incubation with **CyNE 2-DG** or **1**. Cells were incubated with 20 μM **CyNE 2-DG** or **1** at 37°C for 20 min followed by nuclear staining with Hoechst, and fluorescence images were taken under the same acquisition conditions. NIR fluorescence is shown in red color and Hoechst staining in blue color. Scale bar: 100 μm.

5. Cell retention analysis of CyNE 2-DG in MCF7 cells.

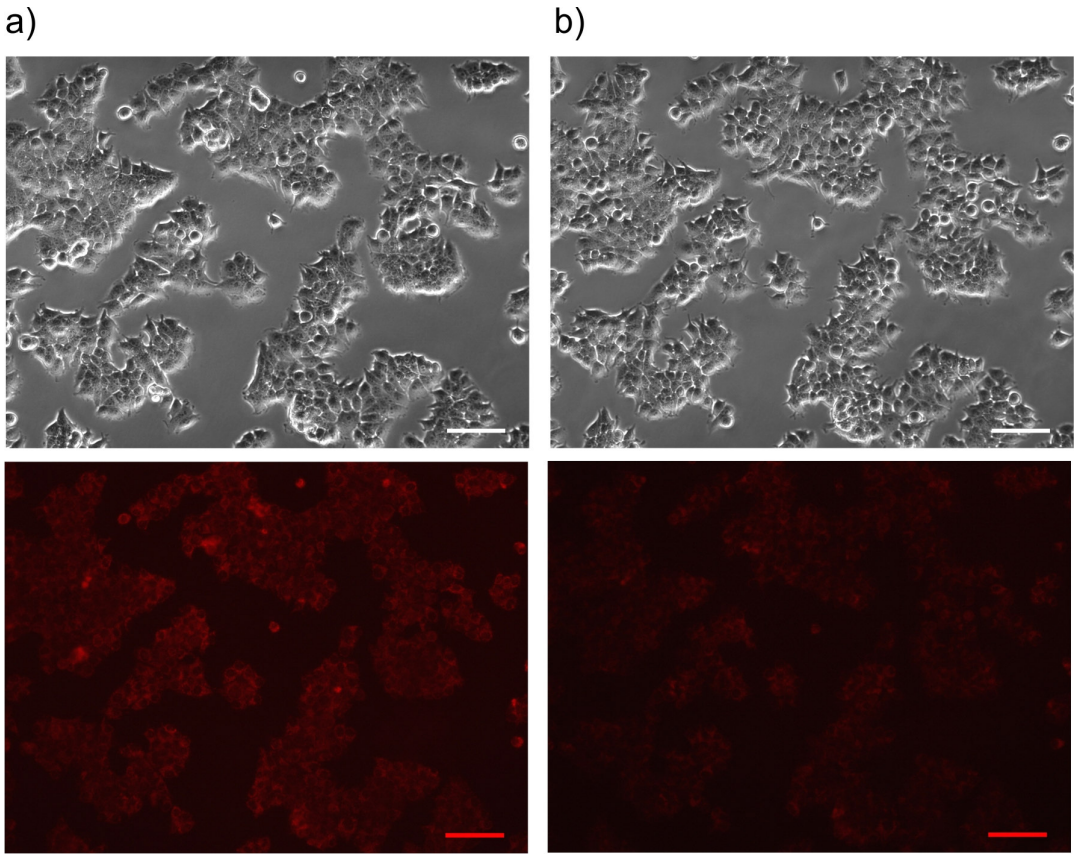

Figure S7. Retention analysis of **CyNE 2-DG** in MCF7 cells upon incubation with 20 μM **CyNE 2-DG** at 37°C for 20 min. Brightfield and fluorescence images were taken under the same acquisition conditions just after treatment (a) and 3 h after treatment with **CyNE 2-DG** (b). Scale bar: 100 μm.