Electronic supplementary information

Red fluorescent luminogen from pyrrole derivatives with aggregation-enhanced emission for cell membrane imaging

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EXPERIMENTAL SECTION

Materials. 4-Bromophenylacetylene, methyl 4-Aminobenzoate and iodine monochloride were purchased from J&K Scientific Ltd. Other chemical reagents such as catalysts and solvents were purchased from Aladdin Industrial Inc. All chemicals were used without further purification. Compounds methyl 4-ethynylbenzoate,[1] 1,4-di(4-bromophenyl)-buta-1,3-diyne (**2**),[2] and 1-methyl benzoate-2,5-di(4-bromophenyl)-pyrrole (**3**)[3] were synthesized according to our previous work. Dimethyl sulfoxide (DMSO), phosphate buffer solution (PBS) and Dlubecco's Modification of Eagle's Medium (DMEM) were purchased from Corning Co. Fetal bovine serum (FBS) was purchased from Gibco Life Technologies. CellTiter 96 Aqueous One Solution Cell Proliferation Assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, MTS) was purchased from Promega Co. Human embryonic kidney cell (293T cell) and human breast cancer cell (MCF-7 cell) were purchased from Beijing Xiehe cell resource center. Triple-distilled water was used for all the experiments.

Instrumentations and Methods. The UV–Vis spectra were recorded on a TU-1901 UV-Vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). Photoluminescence (PL) spectra were collected on a Hitachi F-7000 fluorescence spectrophotometer at room temperature. The nuclear magnetic resonance (NMR) spectra were recorded on a BrukerAMX-400 spectrometer using deuterated chloroform or dichloromethane as solvent. Matrix-assisted laser desorption/ionization time-of-

flight mass spectrometry (MALDI-TOF-MS) was performed by using α-cyano-4-hydroxycinnamic acid (CCA) as the matrix under the reflector mode for data acquisition. PL quantum yields were measured by using an integrating sphere on Nanolog FL3-2iHR fluorescence spectrometer (Horiba Jobin Yvon Company). Confocal imaging was acquired with a Leica TCS SP5 laser scanning confocal microscopy (CLSM). MTS measurement was performed on a BIO-RAD Model 680 enzyme linked immunosorbent assay (ELISA). Cell apoptosis experiments were measured by BECKMAN FC500MCL flow cytometer.

Synthesis of 1-methyl benzoate-2,5-di(4-bromophenyl)-3,4-diiodo-pyrrole (4). Compound **3** (2.0448 g, 4 mmol), $NaHCO_3$ (1.0080 g, 12 mmol), and ICl (1.9482 g, 12 mmol) were mixed together in a 250 mL flask under N_2, and then diethyl ether (30 mL) was added. The reaction mixture was stirred at room temperature for 24 h. The organic solvent was distilled out, and the residual solid was dissolved in dichloromethane and washed successively by saturated aqueous solution of sodium thiosulfate and water for three times. After drying with $MgSO_4$, the solvent was distilled out. The crude product was purified on a silica gel column using hexane and dichloromethane (2:1) as the eluent. A white solid was obtained in 83% yield (2.5330 g). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.82 (d, J = 8.6 Hz, 2H), 7.43 (d, J = 8.5 Hz, 4H), 7.07 (d, J = 8.5 Hz, 4H), 6.93 (d, J = 8.6 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (100 MHz, CD_2Cl_2, δ) 165.72, 141.89, 136.62, 132.53, 131.26, 131.01, 130.08, 129.63, 128.39, 122.51, 52.19. MS (MALDI-TOF, *m/z*): calcd for $C_{24}H_{15}Br_2I_2NO_2$: 762.8; found 763.0.

Synthesis of 1-methyl benzoate-2,5-di{4-{2-[(4-methoxycarbonyl)phenyl] ethynyl}phenyl}-3,4-di{2-[(4-methoxycarbonyl)phenyl] ethynyl}-pyrrole (5). In a three-necked, round-bottomed flask under argon were placed compound **4** (0.7630 g, 1 mmol), methyl 4-ethynylbenzoate (1.2813 g, 8 mmol), dichlorobis(triphenylphosphine) palladium(II) (0.0140 g, 0.02 mmol), triphenylphosphine (0.0105 g, 0.04 mmol), and copper(I) iodide (0.0076 g, 0.04 mmol). Freshly distilled triethylamine (30 mL) and toluene (30 mL) were then added. The resulting mixture was stirred at 65 °C for 16 h. After solvent evaporation, the solid was dissolved in DCM and washed with aqueous solution of NH_4Cl. The organic layer was dried over $MgSO_4$ and then filtered. The

solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography using n-hexane/DCM/ethyl acetate (14:5:1 v/v/v) as eluent. A red solid was obtained in 28% yield (0.2760 g). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.05 (d, J = 8.4 Hz, 3H), 7.82 (dd, J = 8.4, 2.8 Hz, 5H), 7.64 (d, J = 8.3 Hz, 2H), 7.47 (d, J = 8.2 Hz, 2H), 7.37 – 7.29 (m, 3H), 7.24 (dd, J = 8.3, 3.2 Hz, 5H), 6.97 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.3 Hz, 2H), 6.37 (d, J = 8.4 Hz, 2H), 3.92 (m, 15H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 166.61, 166.24, 166.13, 165.87, 144.25, 142.45, 142.18, 142.04, 141.97, 141.29, 140.93, 139.06, 138.93, 133.03, 132.72, 131.34, 131.21, 131.11, 130.94, 130.48, 130.09, 129.85, 129.67, 129.47, 129.30, 128.94, 128.35, 127.77, 127.60, 127.06, 123.58, 122.51, 122.15, 121.98, 121.71, 118.44, 117.42, 103.19, 96.19, 94.57, 94.41, 52.10, 51.85. MS (MALDI-TOF, *m/z*): calcd for $C_{64}H_{43}NO_{10}$: 985.3; found 985.2.

Cell imaging. MCF-7 cell, 293T cell were selected for all experiments. The cell line was maintained in a standard ATCC formulated DMEM supplemented with 10% fetal bovine serum. MCF-7 cells and 293T cells were seeded in a Φ20 mm glass bottom cell culture dish, respectively. After overnight culture, cells were stained with 1.0×10^{-8} mol/L commercial dye DiO (3,3′-dioctadecyloxacarbocyanine perchlorate, membrane staining) for 15 min at 37 ºC, and then washed by using PBS (pH 7.4) solution for three times. After DiO staining, cells were incubated with 1.0×10^{-5} mol/L solution of dye **5** for 10 min at 37 ºC. Before imaging, the cells were washed three times by PBS (pH 7.4) solution. The data of confocal imaging was acquired by CLSM.

Cell viability test. MCF-7 cells were cultured in DMEM (containing 10% FBS) in a humidity incubator at 37 ºC with 5% CO_2 for 24 h. Cells were seeded in 96-well plates at density of 8×10^{3} cells/well. After overnight culture, medium in each wells supplemented with fresh medium containing different concentrations of dye **5**. After 6 h and 24 h treatment, into each well, 20 μL MTS solution was added. After 4 h incubation at 37 ºC, the absorbance of each well at 492 nm was recorded by the enzyme linked immunosorbent assay. Similarly, the cell viabilities of 293T cells were measured.

Annexin V-FITC apoptosis assay. MCF-7 cells were stained by 1.0×10^{-5} mol/L solution of dye **5** for 6 h and 24 h. For apoptosis quantification by Annexin V-FITC/PI

Apoptosis Detection Kit, cells were digested by 0.05% Trypsin and stained with annexin V-FITC and propidium iodide in the dark at room temperature according to the manufacturer's instructions. After treatment at a given time, 400 μL 1× Annexin V binding solution were added into each samples, apoptotic cells were measured by a flow cytometer. Similarly, the apoptotic 293T cells were measured.

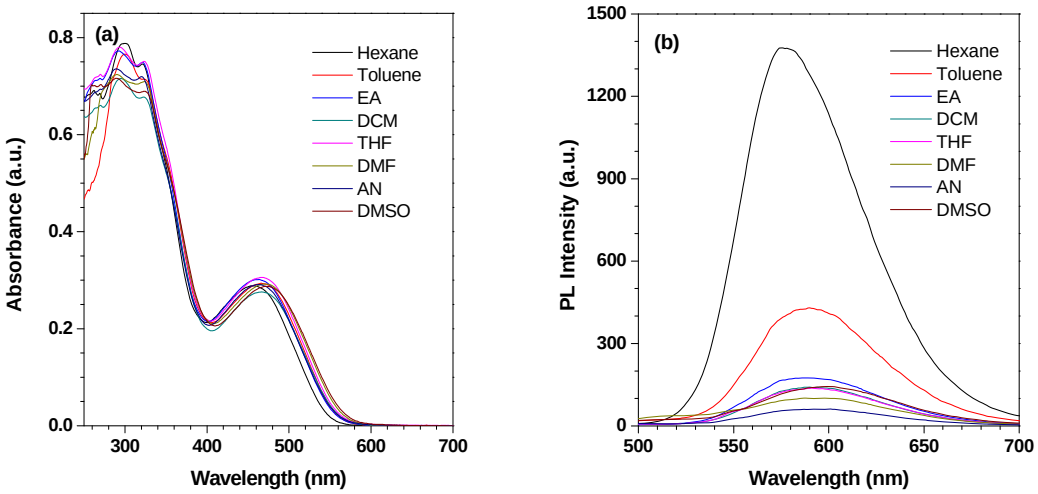

Fig. S1 (a) UV-vis and (b) PL spectra of **5** in hexane, toluene, ethyl acetate (EA), dichloromethane (DCM), tetrahydrofuran (THF), dimethyl formamide (DMF), acetonitrile (AN), dimethyl sulfoxide (DMSO). [**5**]=10 μM; λ_{ex} = 470 nm.

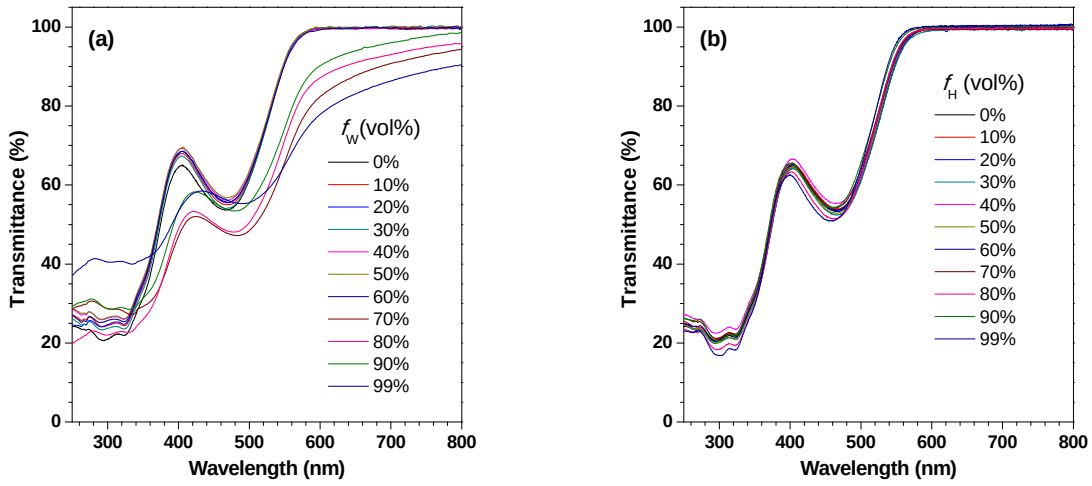

Fig. S2 Transmittance (T%) of **5** in the (a) THF-water and (b) THF-hexane mixtures with different water or hexane fraction. [**5**] = 10 μM.

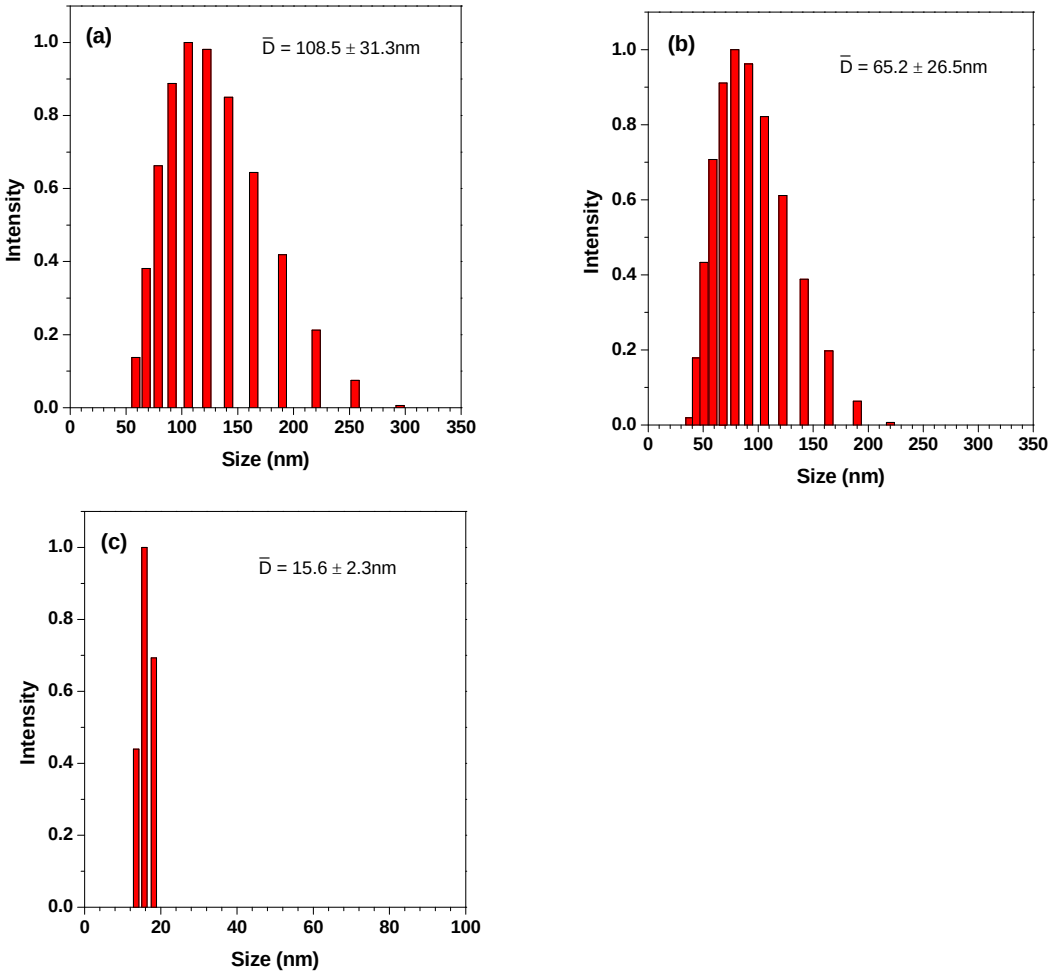

Fig. S3 Dynamic light scattering results of **5** in the THF-water mixtures at (a) 80% and (b) 99% water fraction, and in the THF-hexane mixtures at (c) 99% hexane fraction. [**5**] = 10 μM.

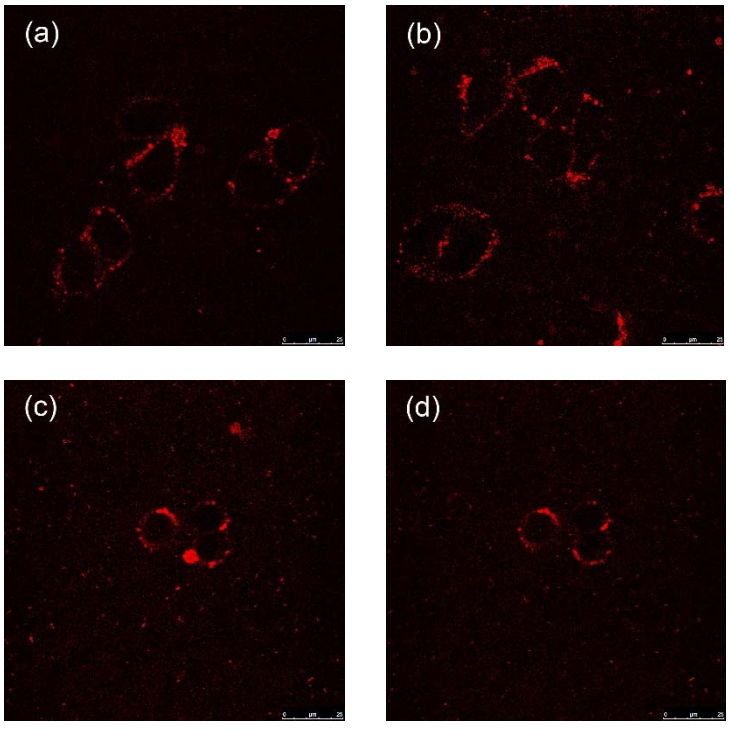

Fig. S4 CLSM images of living MCF-7 (a-b) and 293T (c-d) cells treated with 10 μM solution of dye **5** for 60 min (a, c) and 120 min (b, d) at 37 °C, respectively.

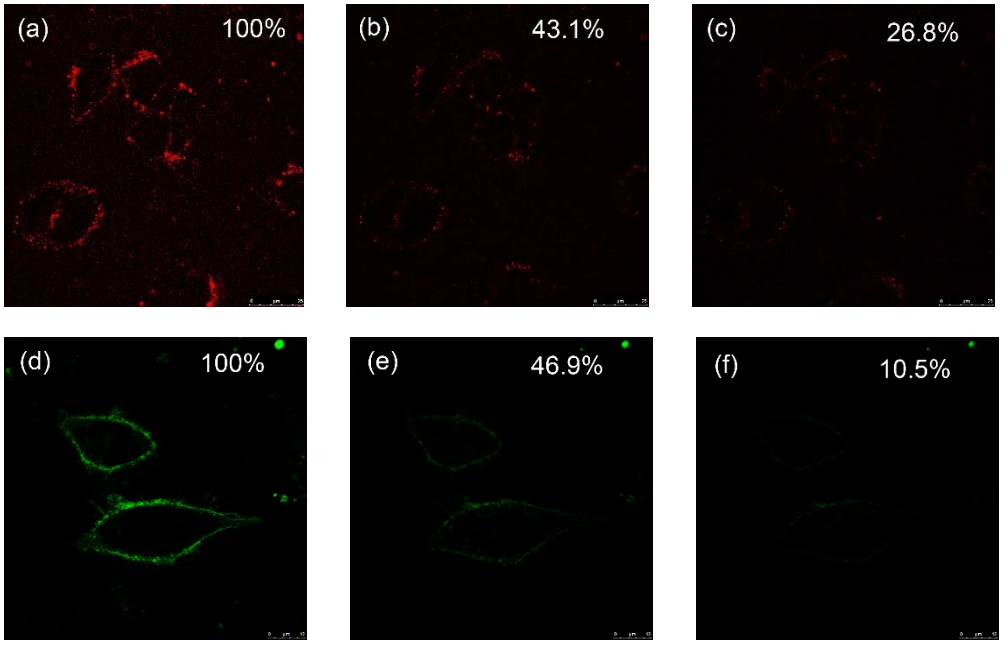

Fig. S5 CLSM images of living MCF-7 cells treated with dye **5** (a-c) and DiO (d-f) in MCF-7 at the different illumination time. 0 h (a, d), 1 h (b, e) and 2 h (c, f). [**5**] = 10 μM; [DiO] = 10 nM.

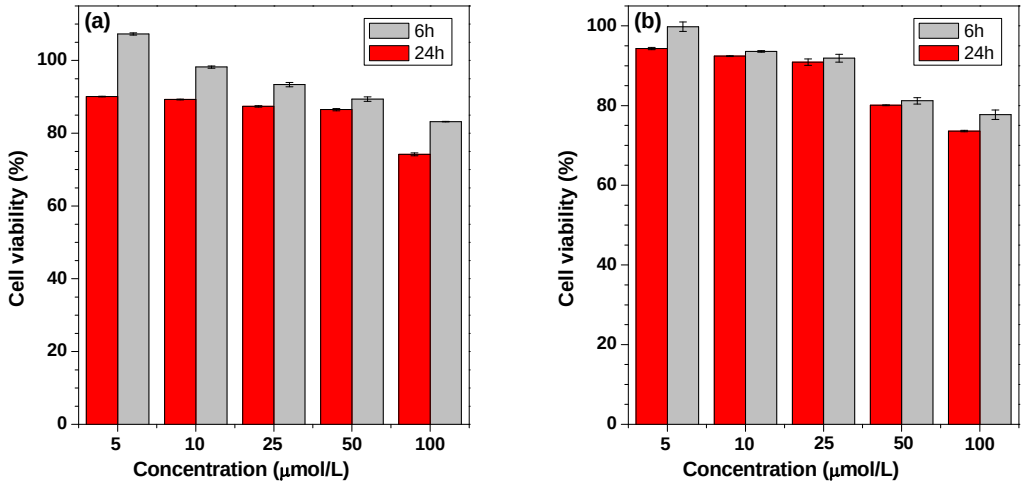

Fig. S6 Biocompatibility evaluations for (a) MCF-7 and (b) 293T cells at different concentrations of dye **5** for 6 and 24h, respectively.

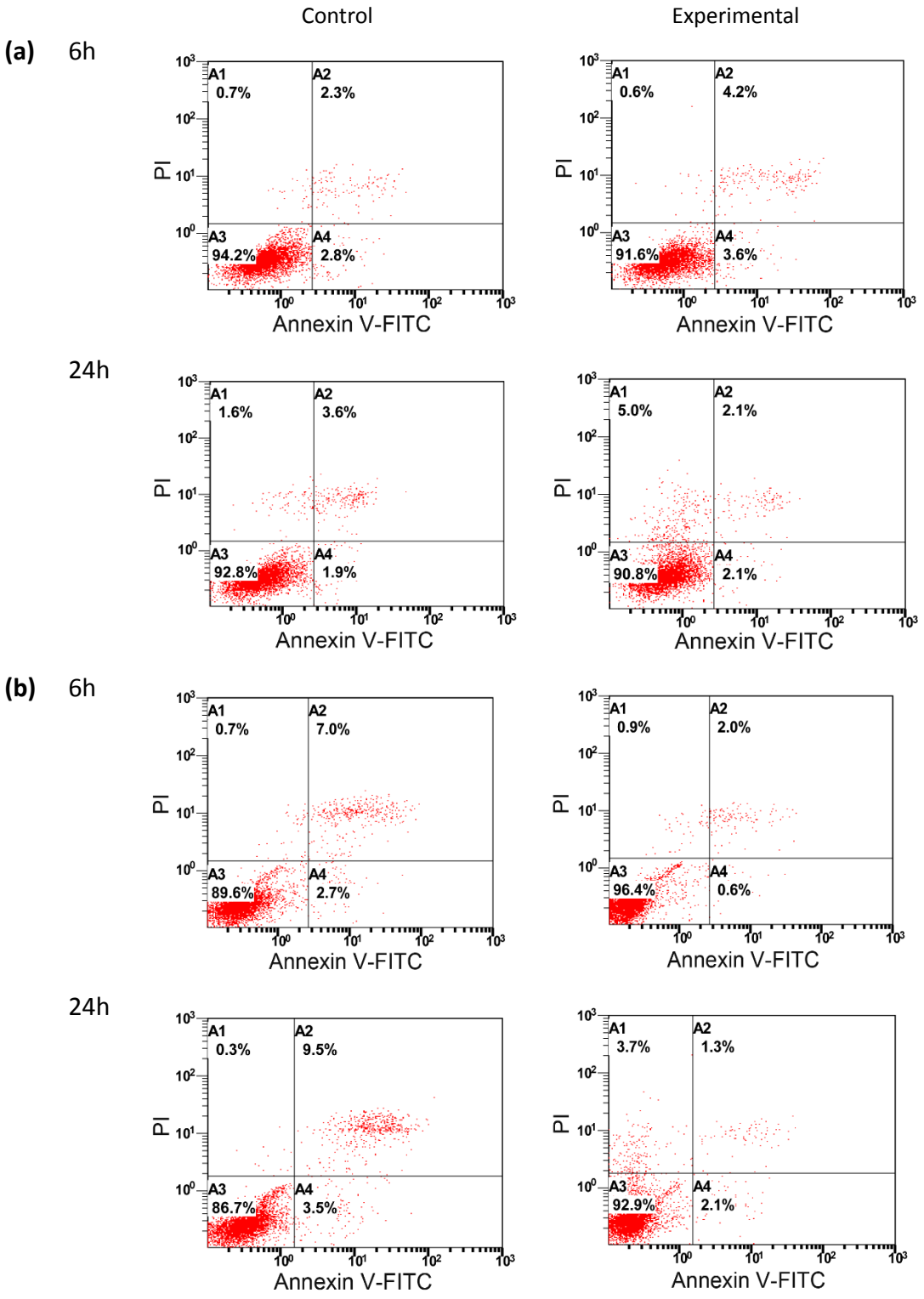

Fig. S7 Evaluation of Apoptosis of (a) MCF-7 and (b) 293T cells treated with 10 μM dye **5** for different time (6 h and 24 h) by using annexin V/PI staining and flow cytometry. Cells are double stained with annexin V-FITC and propidium iodide (PI) and analyzed by flow cytometry. Left: control group; Right: experimental group.

References

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