Supporting information

A Turn-On Near-Infrared Fluorescent Probe for Detection of Cysteine over Glutathione and Homocysteine *in vivo*

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General synthetic procedures.

Compound 1: $POCl_3$ (17.5 g, 115 mmol) was added dropwise to a mixture of DMF (20 ml) and methylene chloride (20 ml) at 0 °C, the mixture was stirred for 30 min at room temperature before the addition of cyclohexanone (5 g, 50 mmol). The resulting solution was stirred for 8 h at 80 °C before it was poured into 50 mL of ice water. The reaction was monitored by thin-layer chromatography (TLC). The reaction solution was filtered and concentrated in vacuo to provide a yellow solid powder, compound **1** (6.3 g, 28.1 % yield), which was used directly in the next step.

Compound 2: 2,3,3-trimethyl-3H-indole (2 g, 12.5 mmol) was dissolved in acetonitrile (50 ml) and stirred for 5 min at room temperature, then 3-bromopropanoic acid (11.47 g, 75 mmol) was added. The mixture was stirred and refluxed for 15 h at 120 °C in dark under nitrogen protection. The reaction was monitored by thin-layer chromatography (TLC). After the reaction, the solvent was removed and a large amount of anhydrous ether was added to recrystallization. The reaction solution was filtered and concentrated in vacuo to provide a light pink solid powder, compound **2** (1.95 g, 14.48 % yield), which was used directly in the next step.

Compound 3: Compound 1 (0.36 g, 2 mmol) and compound 2 (1.17 g, 5mmol) were dissolved ino a mixture of butyl alcohol (20 ml) andtoluene (11 ml) and stirred at 120 °C for 8 h in dark. The reaction was monitored by thin-layer chromatography (TLC). The resulting solution was purified by a large amount of anhydrous ether and concentrated in vacuo to provide a green solid powder, compound **3** (0.96 g, 62.75 % yield), which was used directly in the next step.

Compound Cy-QO: Resorcinol (138 mg, 1.25 mmol) was first added to a mixture of acetonitrile (20 ml) and K_2CO_3 (174 mg, 1.26 mmol) and the mixture was stirred for 10 min at room temperature before the addition of compound **3** (300 mg, 0.5 mmol). The resulting solution was stirred for 4 h at 50 °C and then cooled in room temperature for another 2 h in dark. The reaction was monitored by thin-layer chromatography (TLC). After the reaction, the solvent was removed and the obtained crude product was purified by the silica gel column chromatography (CH_2Cl_2: CH_3OH = 15:1, v/v) to acquire the desired blue-purple solid powder (100 mg, 16.34 %

yield).

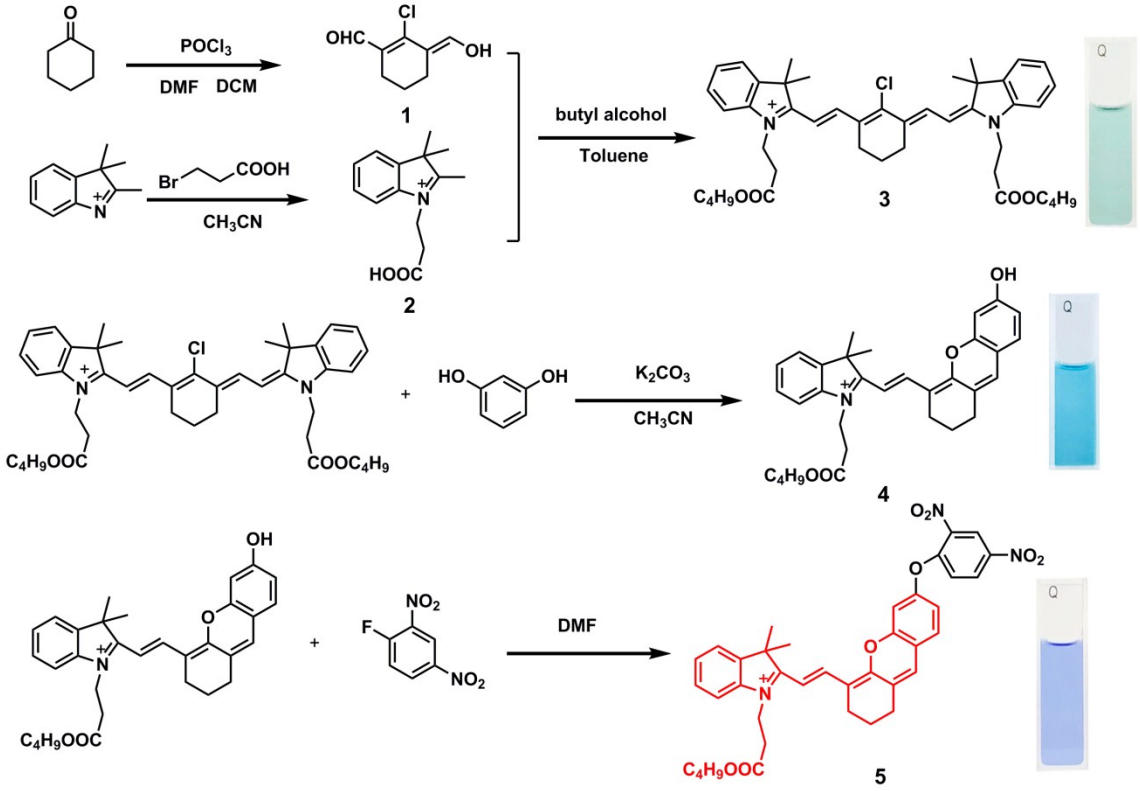

Figure S1. Synthetic route of Probe Cy-Q

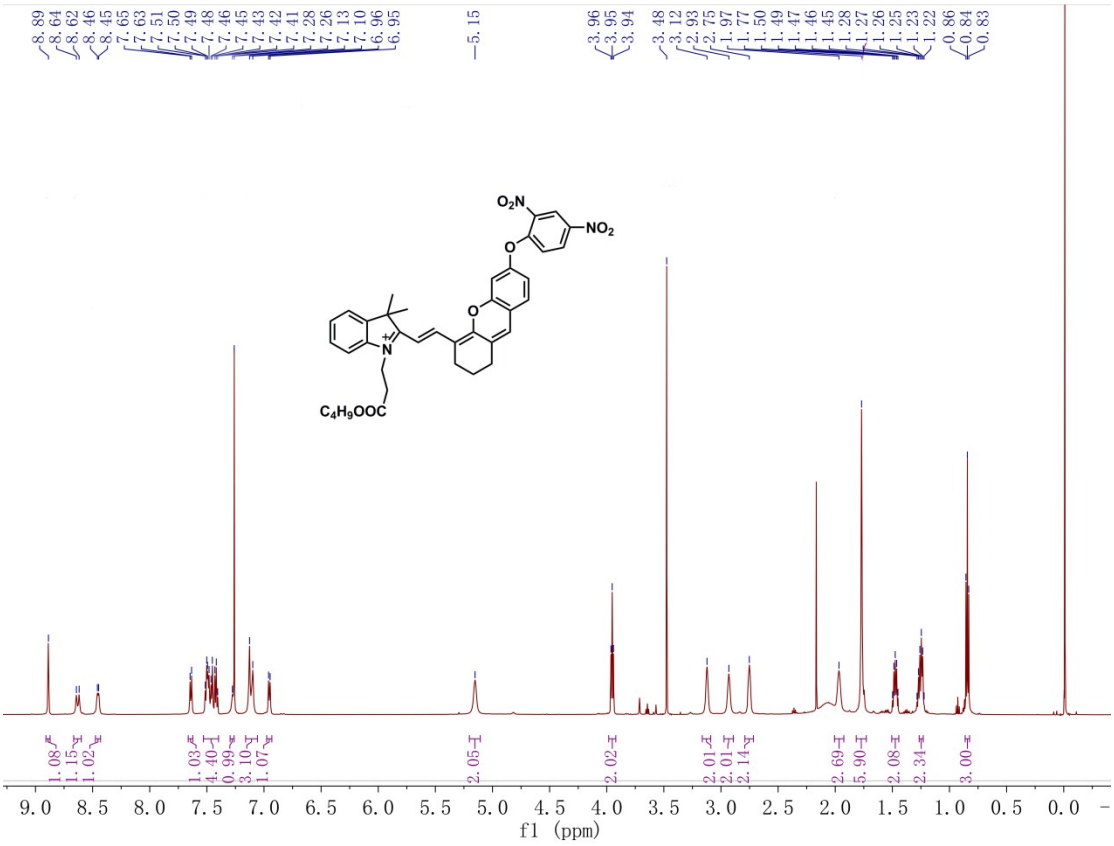

Figure S2. ^{1}H NMR spectra of Cy-Q in CDCl3.

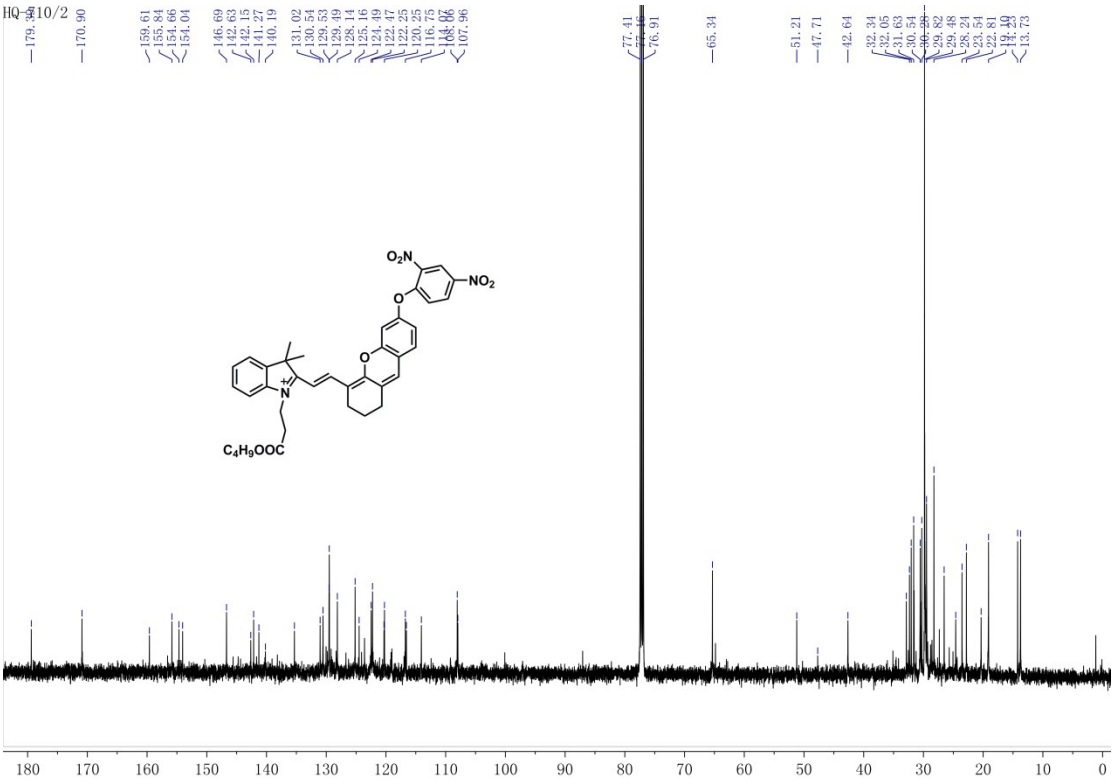

Figure S3. ^{13}C NMR spectrum of Cy-Q in CDCl3.

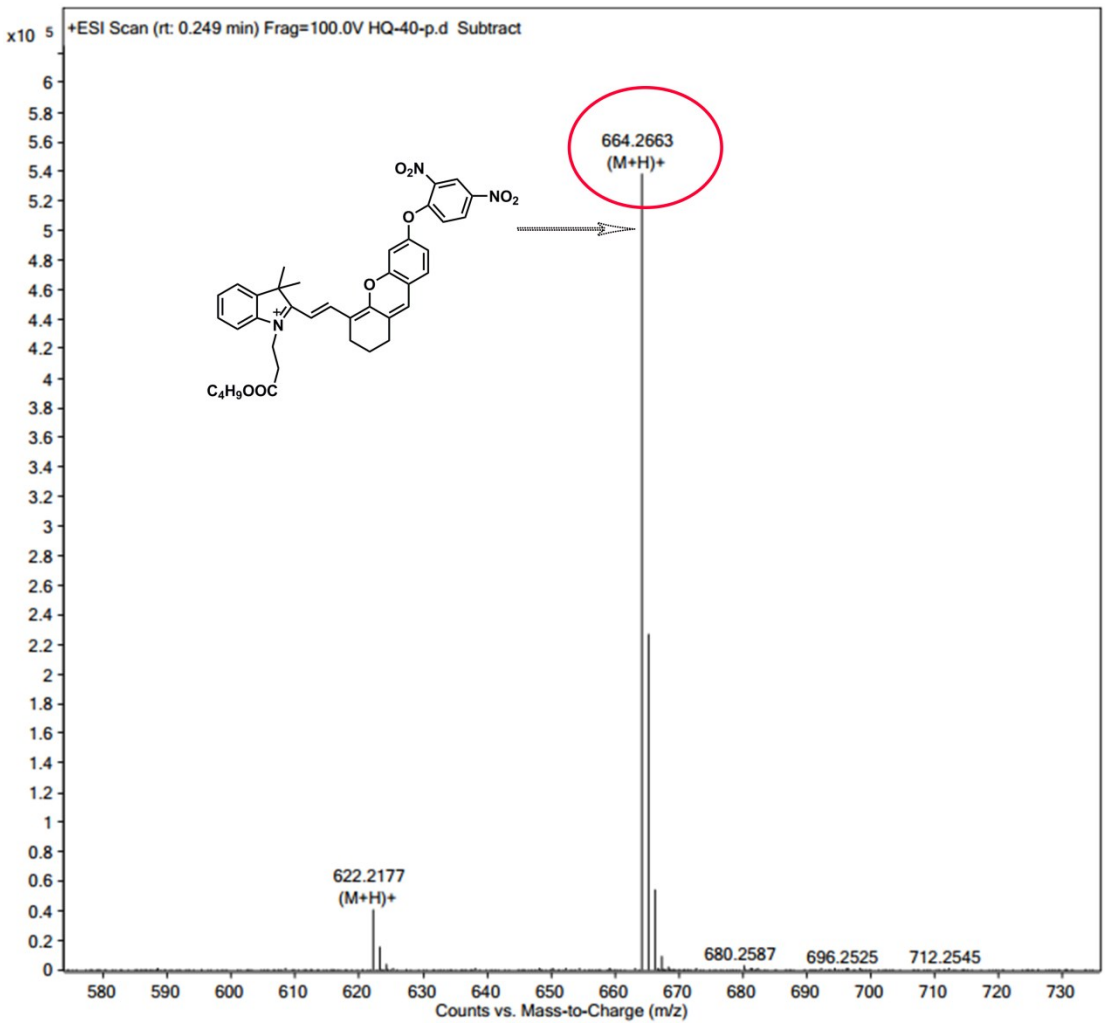

Figure S4. Mass spectrum of Cy-Q.

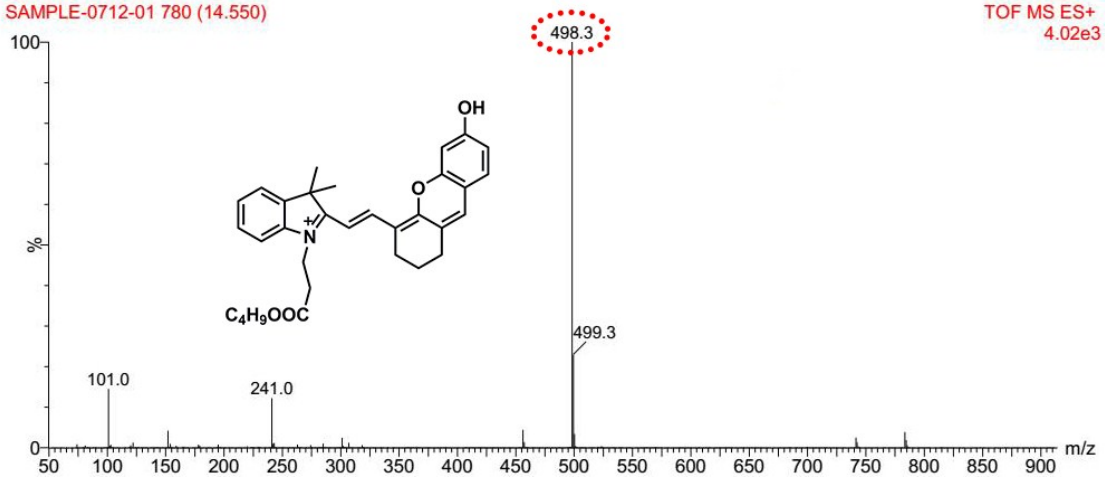

Figure S5. ESI-MS spectrum of Cy-QO after treating probe Cy-Q and Cys.

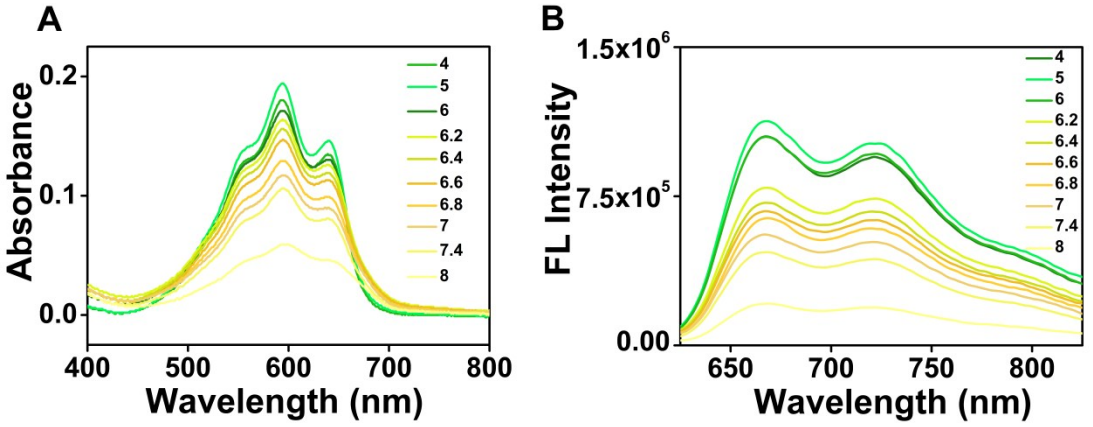

Figure S6. The change of (A) the absorption and (B) fluorescence spectra of Cy-Q (10 μM) in the presence of 10 equiv Cys at different pH (4-8). All experiments were performed in DMSO /PBS buffer (1:99 v/v) at 37 °C. λex = 600 nm.

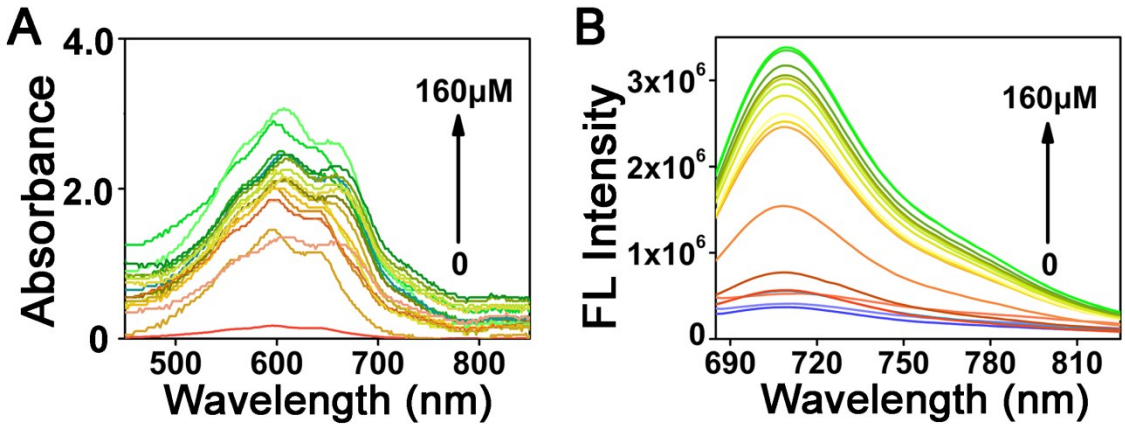

Figure S7. The change of (A) the absorption and (B) fluorescence spectra of Cy-Q (10 μM) with the addition of different concentrations of Cys (0-160.0 μM) in DMSO-PBS buffer (1/99, v/v). λex = 660 nm.

The detection limit of the probe Cy-Q with Cys

The detection limit = 3 ×S.D. / k

Where S.D. represents the standard deviation for the fluorescence intensity of Cy-Q in the absence of Cys, and k is the slope of the curve equation.

The regression equation was y = 2.0405x + 4.38157 with a linear coefficient R^2 of 0.99525 and a standard deviation σ of 0.056.

LOD = 3 ×0.056 / 2.0405 = 82 nM

The limit of detection was calculated as 82 nM.

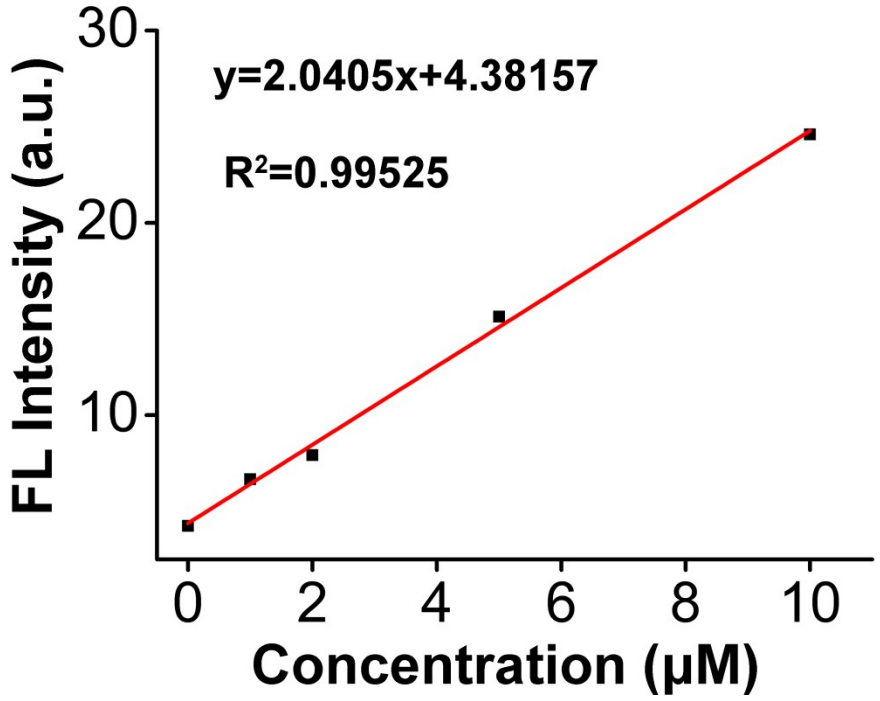

Figure S8. Plot of the intensity at 710 nm for a mixture of Cy-Q (10 μM) and Cys in DMSO–PBS (10 mM, pH=7.4, 1/99, v/v) solution in the range 0-10 μM. λex = 660 nm.

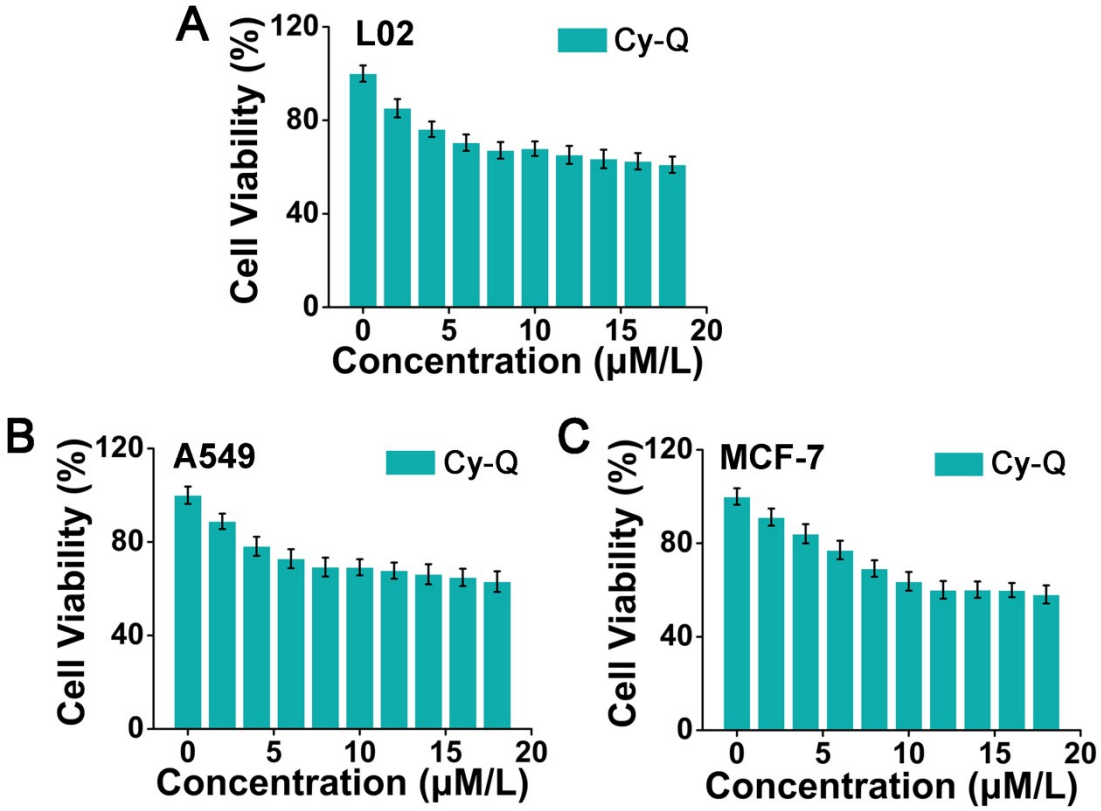

Figure S9. Cell viability of Cy-Q estimated by MTT assay on (A) L02, (B) A549 and (C) MCF-7 cells with an incubation time of 24 h.

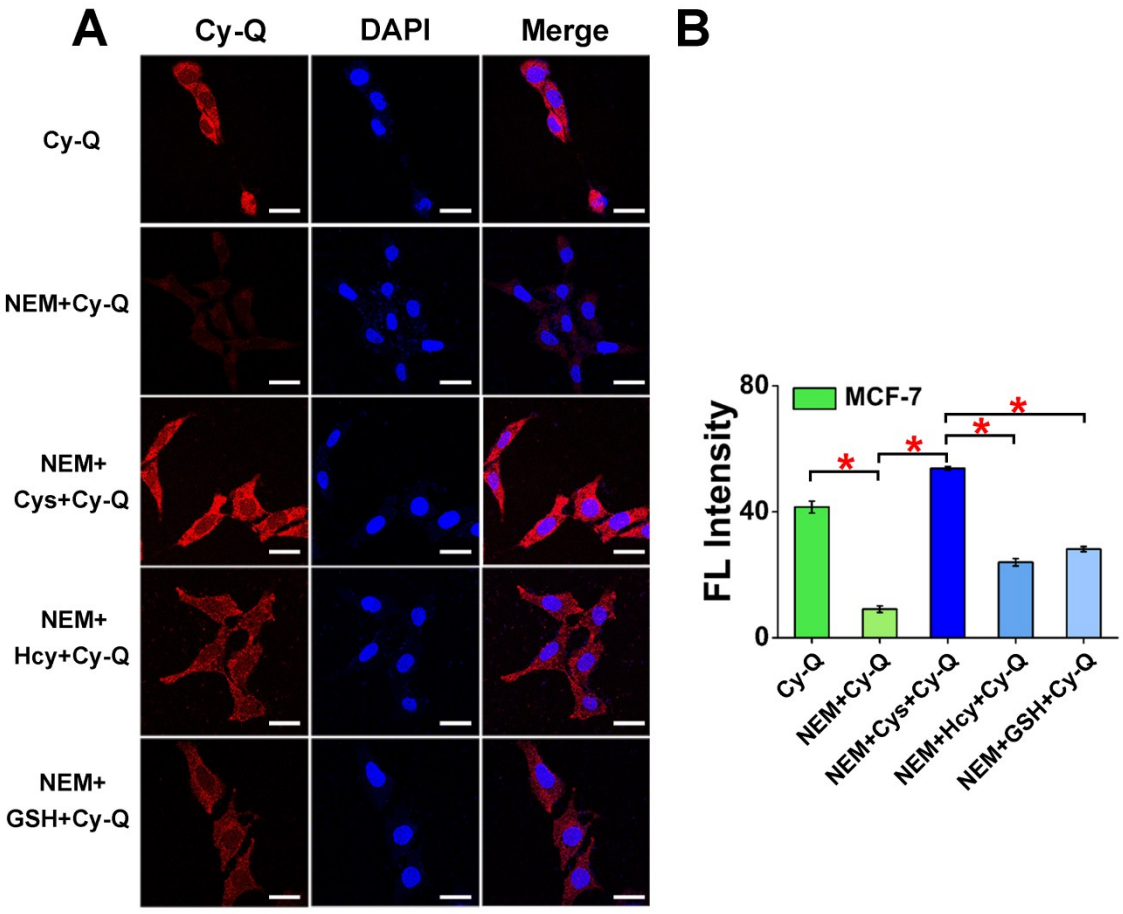

Figure S10. Confocal fluorescence images of Cy-Q in MCF-7 cells. A) Confocal fluorescence images of MCF-7 cells in the presence of Cy-Q (5 μM) only, NEM (1 mM) + Cy-Q (5 μM), NEM (1 mM) + Cys (100 μM) + Cy-Q (5 μM), NEM (1 mM) + Hcy (100 μM) + Cy-Q (5 μM), NEM (1 mM) + GSH (100 μM) + Cy-Q (5 μM). Scale bar: 5 μm; (B) Semi-quantification of the fluorescence intensity of Cy-Q in MCF-7 cells in different groups.

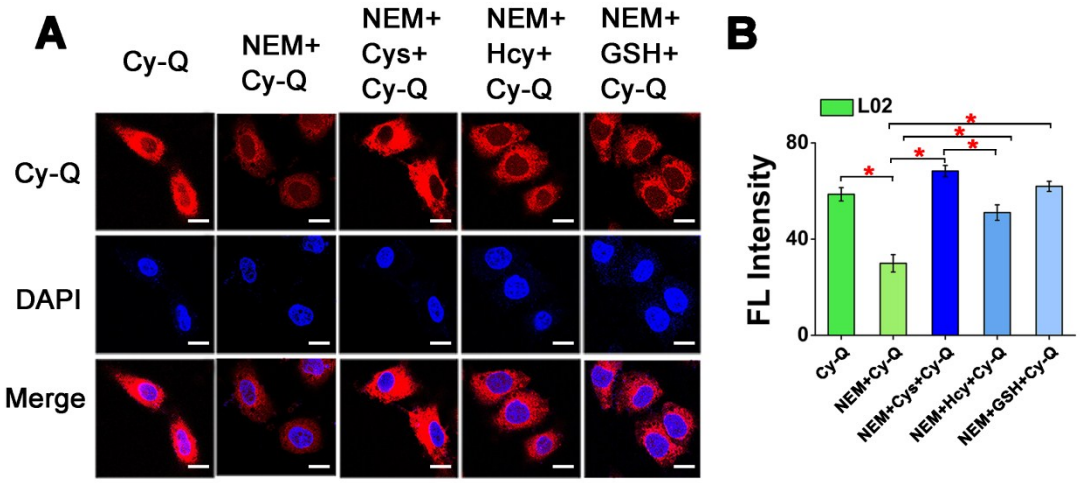

Figure S11. Confocal fluorescence images of Cy-Q in L02 cells. A) Confocal fluorescence images of L02 cells in the presence of Cy-Q (5 μM) only, NEM (1 mM) + Cy-Q (5 μM), NEM (1 mM) + Cys (100 μM) + Cy-Q (5 μM), NEM (1 mM) + Hcy (100 μM) + Cy-Q (5 μM), NEM (1 mM) + GSH (100 μM) + Cy-Q (5 μM). Scale bar: 20 μm; (B) Semi-quantification of the fluorescence intensity of Cy-Q in L02 cells in different conditions.