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Supporting Information

Cell-penetrating peptides modified quantum dots as a ratiometric nanobiosensor for simultaneous sensing and imaging lysosome and extracellular pH

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Experiment section

1. Materials and Reagents

PEG-coated CdSe/ZnS QDs with maximum emission peak of 650 nm was purchased from Xingshuo Nanotech Co., Ltd. (Suzhou, China). RhB-labeled CPPs spirolactam derivative (RhB-R_9SGG(Aib)AAA(Aib)AASL(H_6)) was custom ordered from Top-peptide biotechnology Co., Ltd. (over 95% purity, Shanghai, China). Cell Counting Kit (CCK-8) was supplied by BioDee BioTech Co., Ltd. (Beijing, China). HeLa cells (human cervical cancer cell line), A549 cells (human lung cancer cell line) and MCF-10A cells (human normal breast cell line) were obtained from Peking Union Medical College Hospital (Beijing, China). All the other reagents and solvents were commercially available and used without further purification. Ultrapure water used was purified through a Millipore water purification system (18.2 MΩ.cm@25℃).

2. Characterization Instruments

Zetasizer Nano ZS90 (Malvern, UK) was used to collect Dynamic light scattering (DLS) and zeta-potential data.Fluorescence spectra were recorded on a FP-8600 Spectrophotometer (JASCO, Japan). The absorbance of CCK-8 assay was measured by a microplate reader SpectraMax-M3 (Molecular Devices). Fluorescence imaging experiments were performed on a FV1000 confocal laser scanning microscope (Olympus, Japan) with a 60× objective lens.

3. Preparation of RhB-R_9H_6-QD Nanoconjugates

The peptides were directly dissolved in ultrapure water at a concentration of 1 mM and then dropped into the diluted QDs solution (5 μM). The peptide-to-QD molecular ratio of 160 : 1 produced saturated CPP/QD nanoconjugates according to gel retardation assays, and this ratio was applied in subsequent experiments. The mixture was shaken gently at 25 °C for 2 hours to obtain the nanocomposites.

4. Gel Retardation Assay

Various amounts of RhB-R_9H_6 were mixed with stocks of QDs in PBS at a QDs concentration of 50 nM. These **RhB-R_9H_6-QD** mixtures were incubated at 25 °C for 2 h. The different ratios of **RhB-R_9H_6-QD** (0, 40, 80, 120, 160, 200, 220) were analyzed

by electrophoresis on a 0.8% (w/v) agarose gel in 1× TBE buffer at 100 V for 30 min. The bands on the gel were imaged with UV irradiation at 265 nm.

5. Fluorescence pH Titration

The high concentration of as-prepared **RhB-R_9H_6-QD** nanoconjugates was added to Britton-Robinson (B-R) buffer solutions with varied pH values to a final concentration of 5 μM RhB-R_9H_6 and 30 nM QDs. The fluorescence spectra of samples were obtained on a fluorophotometer with excitation at 559 nm.

6. Cell Culture and Cytotoxicity Tests

HeLa cells and A549 cells were cultured in DMEM supplemented with 10 % FBS and 100 U/mL 1 % penicillin/streptomycin (v/v) in a humidified incubator at 37 °C and 5 % CO2. MCF-10A were grown in DMEM/F12 (1:1) supplemented with 5% horse serum, 10 μg/mL insulin, 20 ng/mL epidermal growth factor. Cell viability was assessed using a CCK-8 assay. Cells were seeded in a 96-well plate in 100 μL of cell medium with a density of 1×10^5 cells / mL overnight, and then incubated with the **RhB-R_9H_6-QD** nanocongugates (RhB-R_9H_6 : QDs = 5 μM : 30 nM) in serum-free medium for 6 h, 12 h and 24 h. Cells were washed and 100 μL fresh medium, 10 μL CCK-8 stock solution were added to each well for another 2 h. The cell viability were evaluated through measurement of the absorbance at 450 nm.

7. Cell Imaging.

The adherent HeLa cells were incubated with **RhB-R_9H_6-QD** nanobiosensor (RhB-R_9H_6 : QDs = 5 μM : 30 nM) in serum-free DMEM for 5 h, followed by washing twice with PBS and loading with fresh DMEM for confocal microscopic analysis. Fluorescence images were collected by Olympus FV1000 confocal laser scanning microscope with a 60× objective lens. The **RhB-R_9H_6-QD** nanobiosensor was excited at 559 nm and the fluorescence emission were collected in the range of 575-630 nm (RhB part, orange channel) and 655-750 nm (QDs part, red channel).

For cellular pH calibration, **RhB-R_9H_6-QD** modified cells were treated with different pH values of high K^+ buffer solution containing 10 μM nigericin for 15 min under standard culture conditions. The calibration curve was plotted on the basis of the average fluorescence intensity ratio of orange and red channel in selected ROIs.

For extracellular acidification tracking, HeLa cells were firstly stained with **RhB-R_9H_6-QD**, then washed and cultured for 0 - 6 h in normal DMEM and DMEM supplemented with additional D-glucose (100 mM.). During the period, confocal imaging was performed at different time intervals and the fluorescence intensity ratio of red and orange channel at the cell surface were recorded. The fluorescence images and pseudocolored ratiometric images were analyzed by using Olympus FV10-ASW software. All data were indicated as mean ± standard deviation.

Fluorescence pH titration in *vitro*

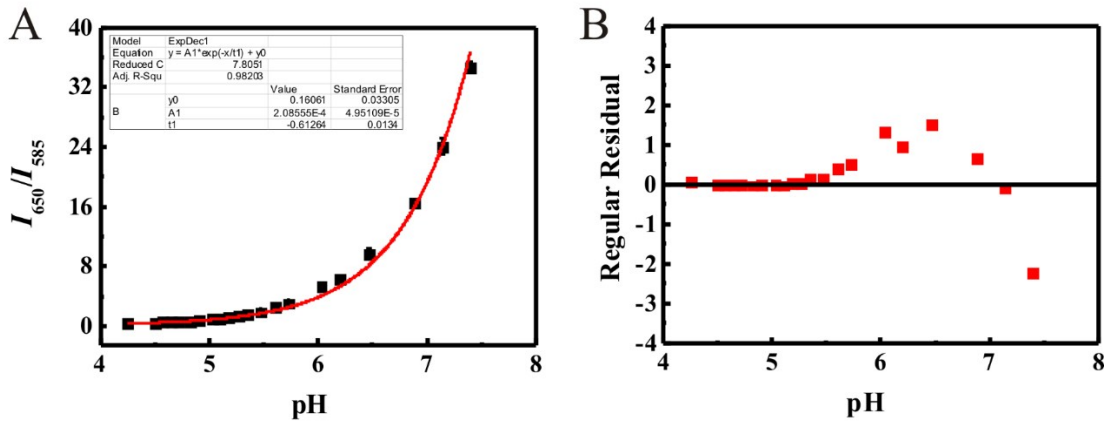

Fig. S1 (A) Plot of I_{650}/I_{585} vs pH values. (B) The residue plot.

Cytotoxicity assay

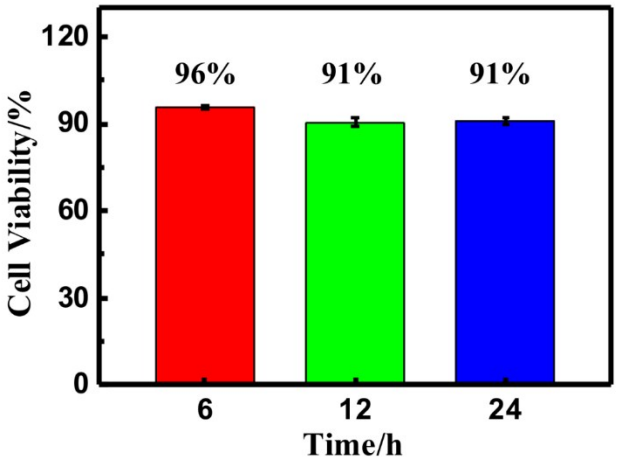

Fig. S2 The viability of Hela cells incubated with **RhB-R_9H_6-QD** for 6 h, 12 h and 24 h. The error bar represent standard deviations based on three independent measurements.

Z-axis scanning confocal images

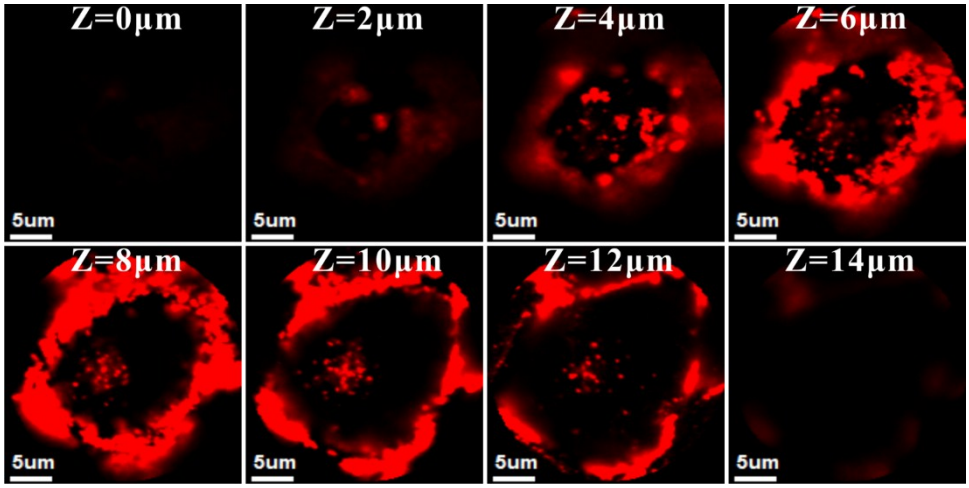

Fig. S3 Z-axis scanning confocal images of **RhB-R_9H_6-QD** modified HeLa cells.

Confocal images of Trypan blue quenching experiment

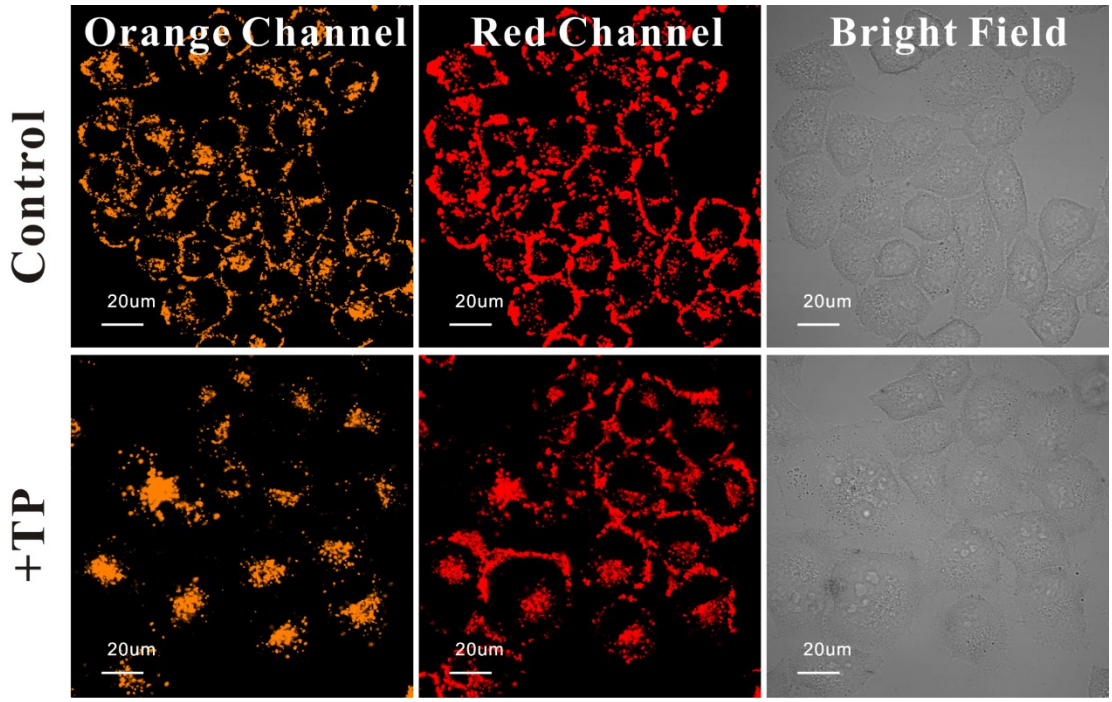

Fig. S4 RhB-R_9H_6-QD modified HeLa cells treated with 20 μg/mL Trypan blue.

Process of cell labelling with RhB-R_9H_6-QD

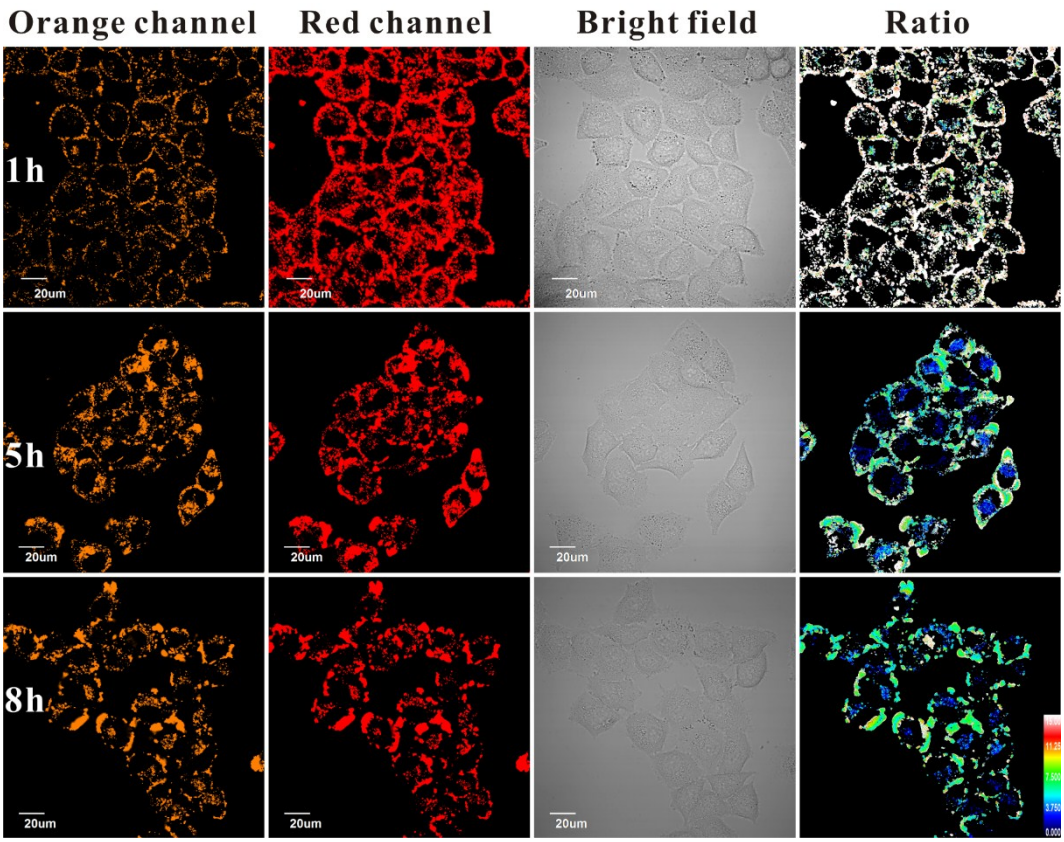

Fig. S5 Confocal and ratio images of HeLa cells incubated with **RhB-R_9H_6-QD** for different time.

Confocal images co-localization experiment

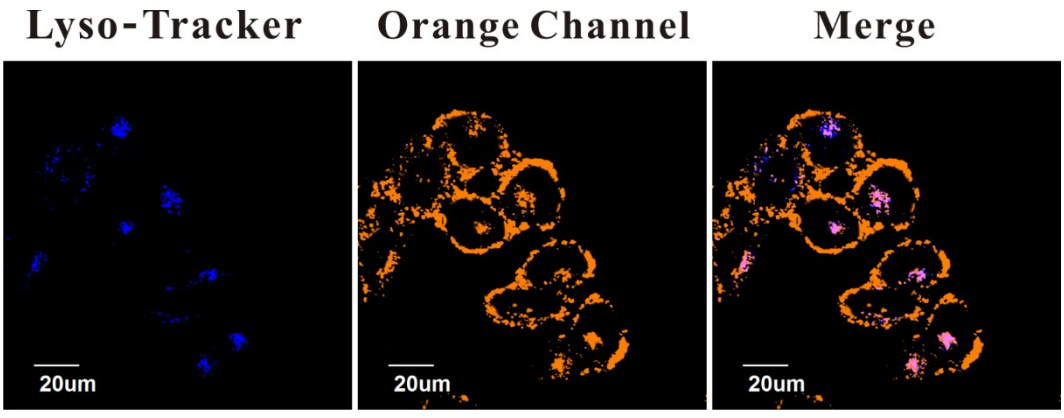

Fig. S6 Images of HeLa cells costained with **RhB-R_9H_6-QD** and LysoTracker DND-22.

Confocal images of different cell types modified with RhB-R_9H_6-QD

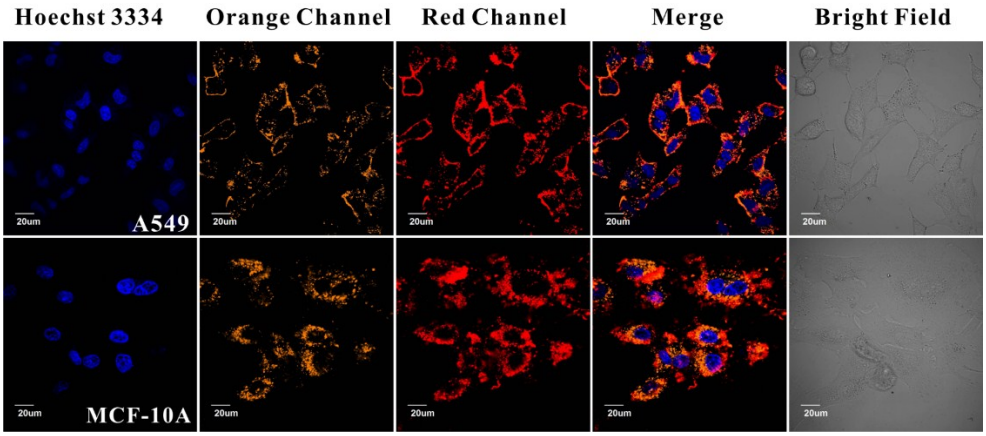

Fig. S7 Confocal images of different cell types modified with **RhB-R_9H_6-QD**.

Fluorescence ratiometric pH sensing of living cells

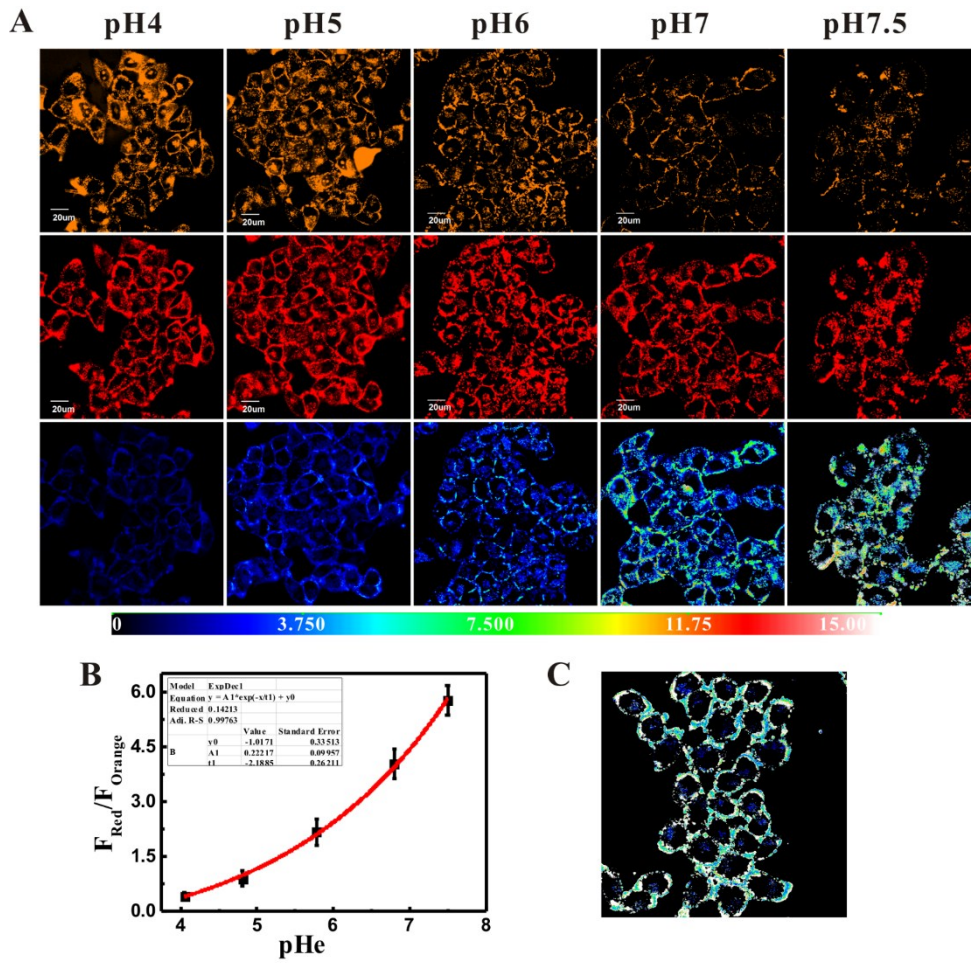

Fig. S8 (A) Confocal and ratio images of **RhB-R_9H_6-QD** modified HeLa cells exposed to external media at pH 4, 5, 6, 7 and 7.5. (B) Plot of fluorescence intensity ratio vs pHe. (C) Ratio image of HeLa cells modified with **RhB-R_9H_6-QD** in complete medium.

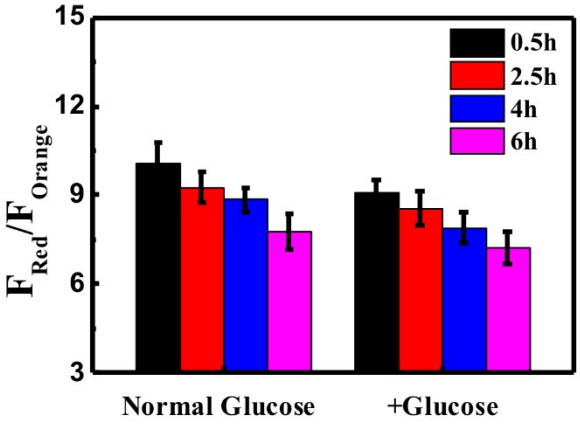

Fig. S9 The alteration of extracellular pH of **RhB-R_9H_6-QD** modified HeLa cells in normal and high glucose metabolism. The error bar represent standard deviations based on fluorescence intensity ratio extracted from at least 15 individual ROIs.