Supporting Information for：

SERS Monitoring the Dynamics of Local pH in Lysosome of Living Cells during Photothermal Therapy

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EXPERIMENTAL SECTION

Materials and instruments. The peptide (GRRRRRRGKFFFC) was synthesized by Chutai Biotechnology Co. Ltd. (Shanghai, China). Ultrapure water obtained from a Millipore water purification system (18 M Ω) was used in all assays. N-cetyltrime thylammonium bromide (CTAB), (3-aminopropyl) triethoxysilane (APTES), succinic anhydride, silver nitrate ($AgNO_3$), auric chloride acid ($HAuCl_4.4H_2O$), sodium boron hydride ($NaBH_4$) and ascorbic acid were purchased from J&K Scientific. All chemicals were analytical grade and used as received without further purification. Human cervical carcinoma cancer cell (HeLa cell) was obtained from the American Type Culture Collection (Manassas, VA). Transmission electron microscopy (TEM) images were obtained with a JEOL-3010 using an accelerating voltage of 100 kV. Zeta potential experiments and dynamic light scattering (DLS) measurements were performed at room temperature using a Malvern Zeta Sizer Nanoseries (Nano ZS90). UV-Vis spectra were collected using a Hitachi U-4100 spectrophotometer (Kyoto, Japan). All fluorescence measurements were performed on a PTI Fluorescence System (Photo Technology International, Birmingham, NJ). Confocal laser scanning microscopy (CLSM) images were obtained on a Fluoview TM FV 1000 (Olympus, Japan). MTT assay was obtained on a Synergy™ 2 Multi-Mode Microplate Reader (Bio-Tek, Winooski, TV).

Synthesis of AuNRs. AuNRs were prepared according to the seed-mediated protocol using CTAB as a soft template. Briefly, in the presence of 100 mM CTAB solution, gold seeds were prepared by reducing 300 μM $HAuCl_4.4H_2O$ with 10 μM ice-cold $NaBH_4$. During vigorous stirring, the mixture rapidly developed a light brown color and the solution was then kept at room temperature before further use. A 50 mL growth solution was prepared containing $HAuCl_4.4H_2O$ (300 μM) and CTAB (100 mM), followed by the addition of 0.2 mL of 10 mM $AgNO_3$ and 0.2 mL of 200 mM

L-ascorbic acid. During mixing, the solution immediately became colorless. Finally, 0.2 mL of 3 h-aged gold seed solution was added to the above solution and stirred vigorously for 60 s, with the color gradually becoming brown. The mixed solution was left undisturbed overnight for further growth.

Preparation of AuNR@4-MBA and AMP. The AuNRs were subsequently functionalized with 4-MBA to obtain the AuNR@4-MBA by adding 200 μL of 10 mM 4-MBA aqueous solution dropwise to 20 mL of as-prepared AuNRs solutions under vigorous stirring. Then, 200 μL 5 mM peptide aqueous solution was added dropwise to the obtained AuNR@4-MBA pH nanotracker solutions under vigorous stirring for overnight to obtain the AMP pH nanotracker. The conjugation of TAMRA-labeled peptide (GRRRRRRGKFFFCKFFFCKFRRRRGRRRRRGGK) on the AuNR@4-MBA surface was determined by fluorescence measurement of fluorophore TAMRA. The fluorescence maxima of the supernatant, containing free peptide removed from the particle, were converted to molar concentrations of the TAMRA modified peptide by interpolation from a standard linear calibration curve. Standard curves were prepared with known concentrations of TAMRA-labeled peptide using identical buffer pH and salt concentrations. The concentration was estimated to 53 peptide on each particle. For the SERS measurements, 1.0 mL aliquots of the pH nanotracker solution were added to Eppendorf tubes, and each tube was centrifuged at 8000 rpm for 5 min and the supernatant was then removed and discarded. After that, the pH nanotracker was mixed with 500 μL of HEPES buffer and then stored in Eppendorf tubes for SERS measurements.

Effect of Laser-Induced Temperature Change. The photothermal property of AMP were subsequently verified through a temperature elevation experiment using a 780 nm laser as the irradiation source. AuNR and AMP were at the concentration of 200 μg/mL, NIR laser irradiated at 2.0 W/cm^2 for different time. HeLa cells incubated with AMP were treated with trypsin, centrifuged at 2000 rpm for 5 minutes to remove trypsin and cell culture medium, then re-dispersed in HEPES before temperature measurement.

Cell Culture and Viability Assay. The cytotoxicity was investigated by MTT assay for HeLa cell lines in a 96-well. HeLa cells (5×10^4 cell per well) were treated with serial concentrations of AuNR@4-MBA and AMP. For PTT, the cells were irradiated with 2.0 W/cm^2 NIR laser (780 nm). After irradiation, cells were then incubated at 37 ^{o}C under 5 % CO_2 for 12 h, during which cells grew in log phase. Finally, 60 μL MTT

solution (1 mg/mL) in DMEM medium solution was added to each well and incubated at 37 ^{o}C for 2 h. The precipitated formazan violet crystals were dissolved in 200 μL of DMSO. The absorbance of formazan was measured at 570 nm by ELISA reader. Each experiment was repeated at least three times.

Lysosome pH Calibration. HeLa cell lines were grown in DMEM cell medium with 10% inactivated fetal bovine serum at 37°C in a humidified incubator containing 5% CO_2. After removing the medium, cells were incubated with fresh medium containing AMP (0.4 nM, particle concentration was determined by AuNRs, concentration of AuNRs was calculated using Lambert-Beer's law according to the following equation: $A=\varepsilon bc$, where the extinction coefficient (ε) is $1.9\pm0.4\times10^{9}$ $M^{-1}cm^{-1}$ for λ=695 nm AuNRs and b = 1 cm (for standard cuvettes)) for 3 h. Then cells were washed three times by washing buffer (WB; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 5 mM $MgCl_2$, and 250 mM glucose, pH 7.4) and treated with high K^{+}-HEPES-buffer solution (20 mM NaCl, 125 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM glucose, and 20 mM HEPES) at various pH values (pH 4.5, 5.5, 6.0, 6.5 and 7.4) in the presence of 10.0 μM nigericin. After 20 min, cells were used for SERS imaging.

Determination of pH during PTT in Lysosome of Living Cells. HeLa cell lines were seeded on 35 mm glass bottom dishes and grown for 48 h prior to treatment. For lysosome localization experiment, HeLa cells were incubated with fresh media containing AMP (0.4 nM) for 3 h. Then, the cells were washed to remove excess AMP by WB and incubated with 2 mL binding buffer (BB, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 5 mM $MgCl_2$, 250 mM glucose, 0.1 mg/mL tRNA, and 1 mg/mL BSA, pH 7.4) containing 0.5 μL of Lyso Tracker Blue for 15 min. Subsequently, the cells were washed three times, resuspensed in 2 mL of BB, and imaged on a laser-scanning confocal microscope. The Pearson's correction coeffcient was measured by software Image Proplus 6.0. For the drug stimuli experiment, HeLa cells were first incubated with fresh medium containing AMP (0.4 nM) for 3 h (Subsequently, the cells were washed to remove excess AMP, resuspensed in 2 mL of BB)and then treated with 200 μM CQ for 40 min and 1 μM DEX for 6 h at 37 °C, respectively. Then the cells were washed three times by WB and used for SERS imaging. PTT-triggered pH measurement data were generated by incubating HeLa cells with AM P for different illumination time and then used for

SERS imaging.

Lysosomal Integrity Assays. Confocal fluorescence images of HeLa cells after treatment with HEPES buffer, AMP incubation, and then laser exposure were stained with 5.0 μM AO for 15 min to perform confocal fluorescence imaging. The images are collected from 515 to 545 nm (green) and 610 to 640 nm (red) at an excitation wavelength of 488 nm.

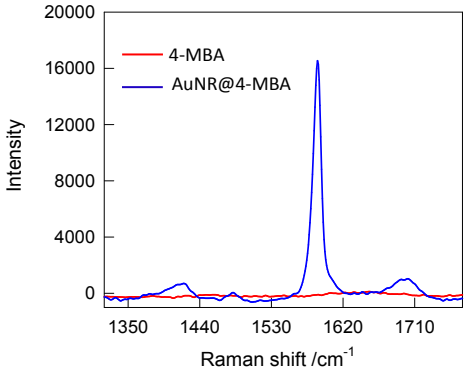

Figure S1. SERS spectra of free 4-MBA and AuNR@4-MBA in 10 mM HEPES solution (pH 7.4).

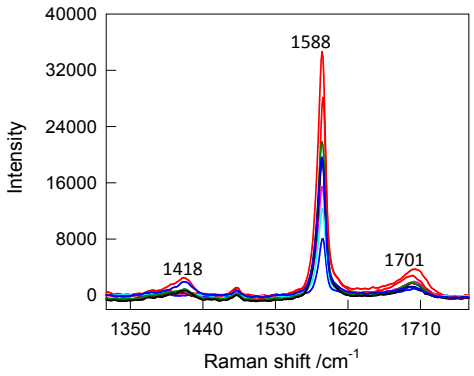

Figure S2. SERS spectra of AMP in 10 mM HEPES solutions of various pH values ranging from pH 4.5 to 8.5 in a step of 0.5 pH unit with illumination (780 nm, 2.0 W/cm^2, 20 min).

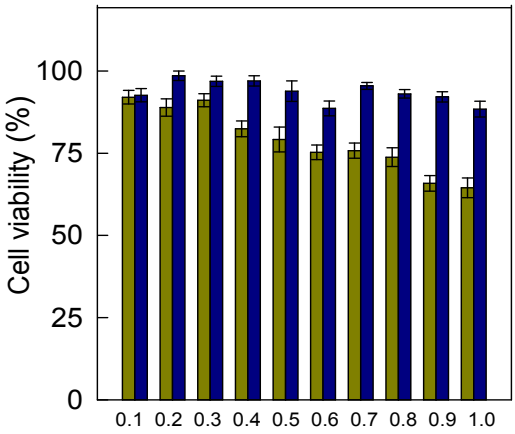

Figure S3. Cytotoxicity assay of HeLa cells treated with AuNR@4-MBA and AMP. The concentration of AuNR@4-MBA (grass green bar) and AMP (blue bar) were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 nM, respectively.

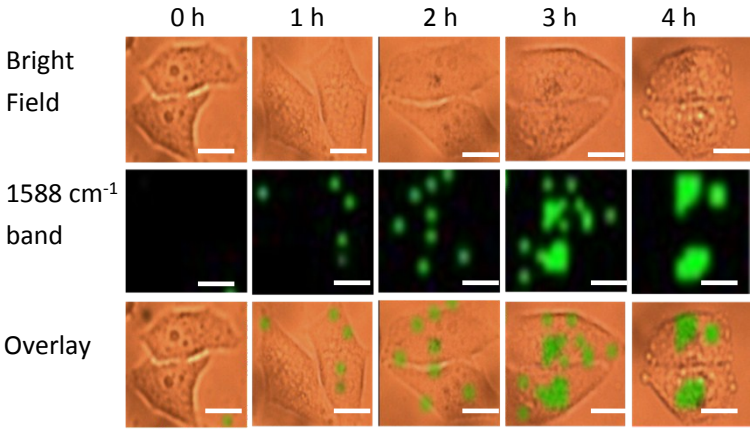

Figure S4. SERS imaging of AMP-treated HeLa cells as a function of different incubation time. Scale bar: 10 μm. [AMP]=0.4 nM.

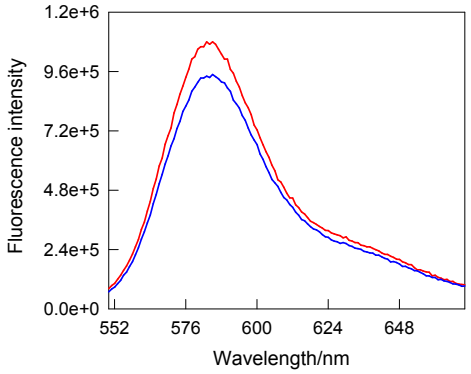

Figure S5. Fluorescence spectrum of TAMRA-labeled peptide before (red curve) and after (blue curve) conjugated to the surface of AuNR@4-MBA@PEG-SH. [Peptide]= 2 μM. The concentration of AuNR@4-MBA was excessive. λex= 520 nm.

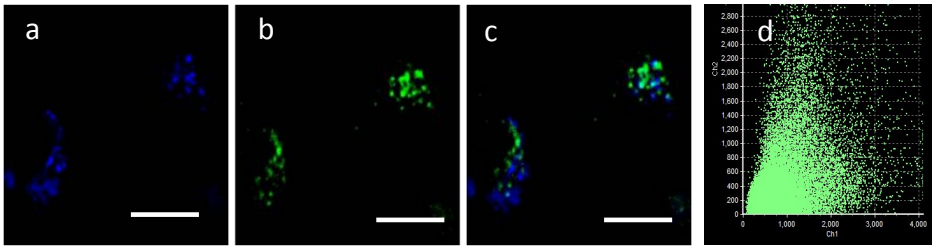

Figure S6. Co-localization experiment involved the fluorescent AMP and Lyso Tracker Green in HeLa cells. (a) Fluorescence imaging of TAMRA in HeLa cells; (b) Fluorescence imaging of Lyso Tracker Green in HeLa cells; (c) Overlay of a and b; (d) Pearson's coffents (measured by software Image Proplus 6.0) were 0.775, respectively. [AMP]=0.4 nM. Scale bar: 10 μm.

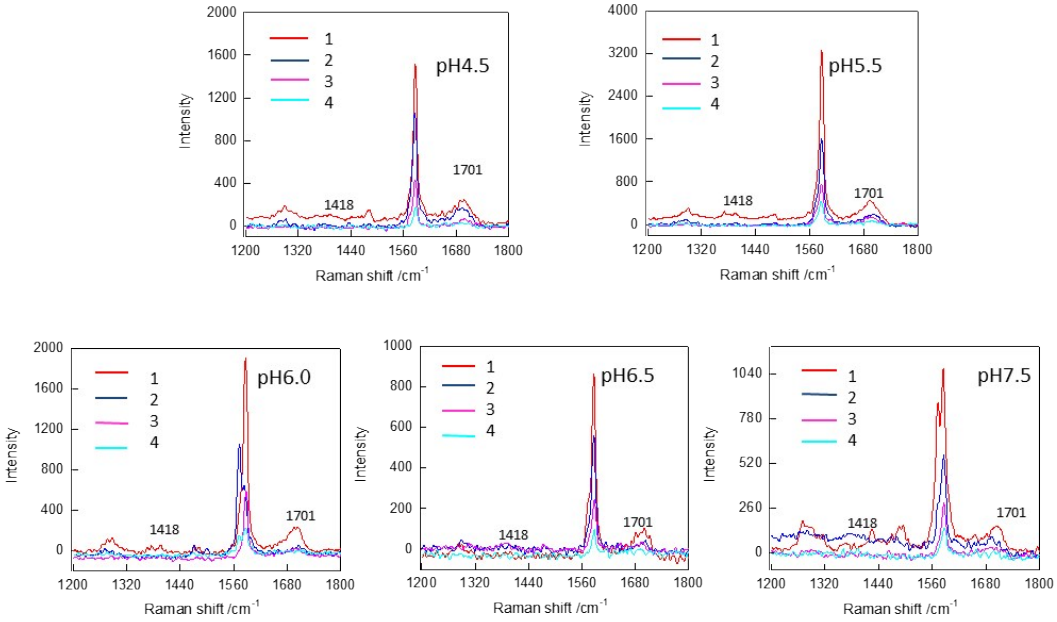

Figure S7. The corresponding SERS spectra of Figure 3A (b1-b5).

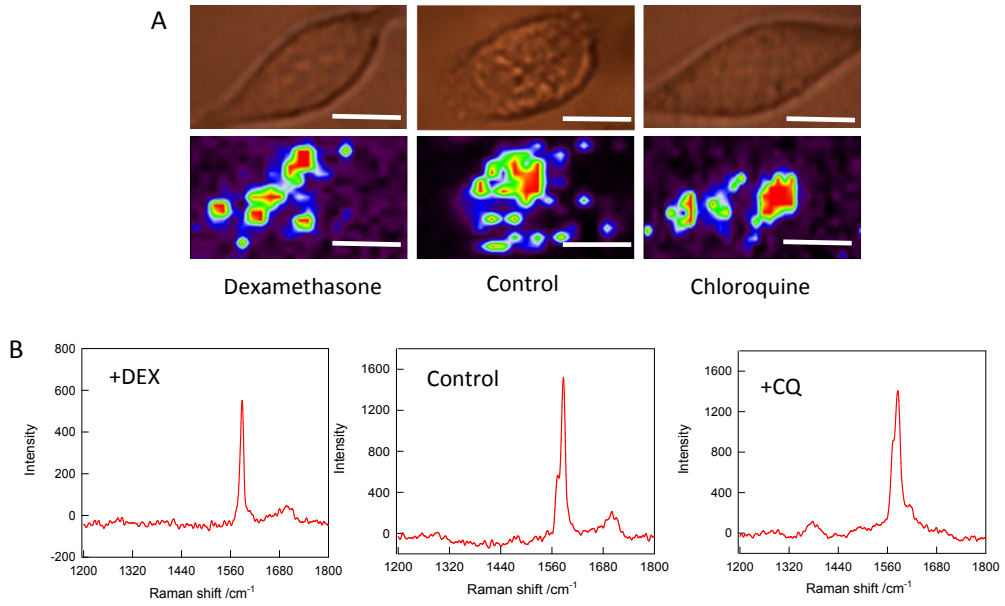

Figure S8. (A) SERS imaging analysis of AMP-incubated HeLa cells treated with stimuli chloroquine (CQ), dexamethasone (DEX) and the untreated control group. (B) The corresponding representative SERS spectra of (A). [AMP]=0.4 nM. Scale bar: 10 μm.

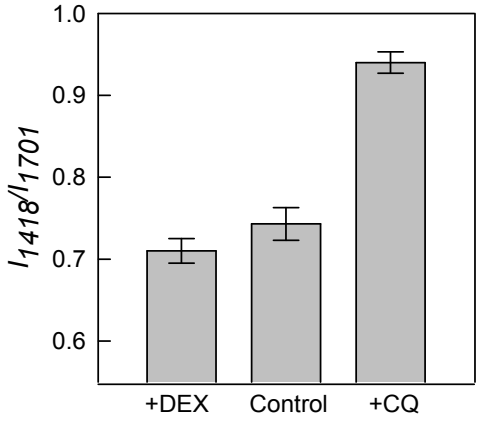

Figure S9. Histogram of I_{1418}/I_{1701} of HeLa cells treated with stimuli chloroquine (CQ), dexamethasone (DEX) and the untreated control group. Error bars represent the standard errors of the mean.

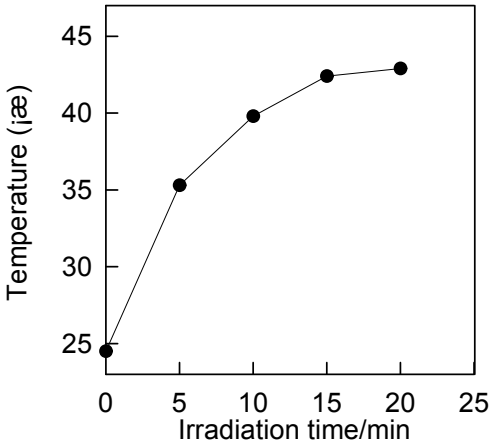

Figure S10. Temperature-time curve of HeLa cells upon treated with AMP followed by 780 nm laser irradiation at the dose of 2.0 W/cm^2.

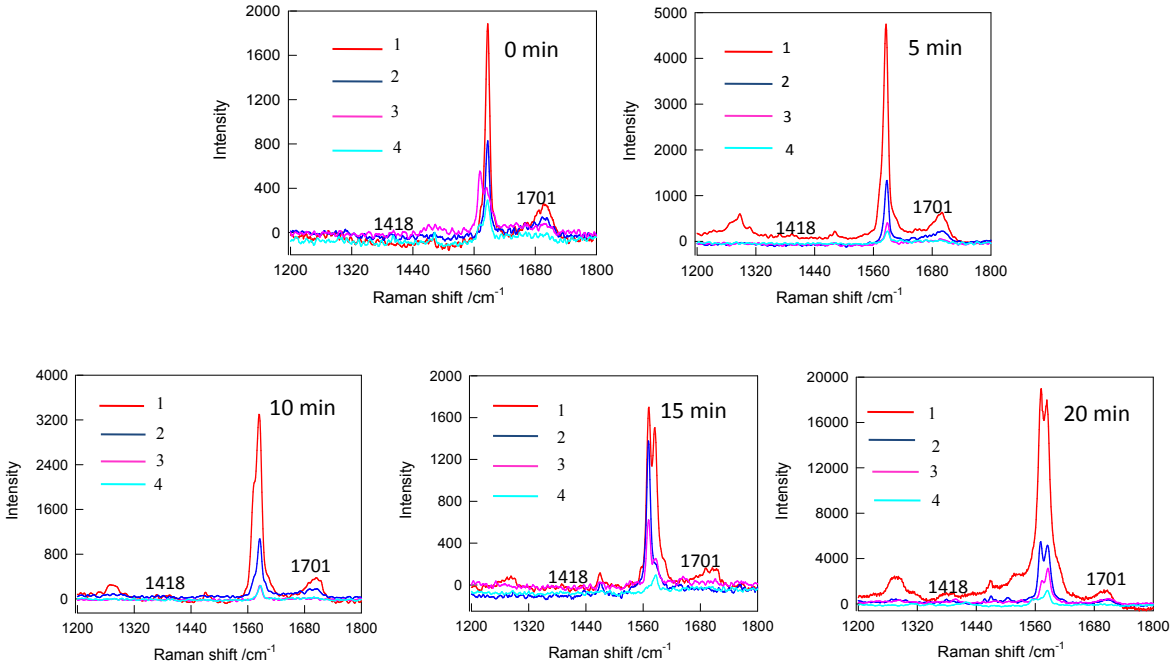

Figure S11. The corresponding SERS spectra of Figure 4A (b1-b5).

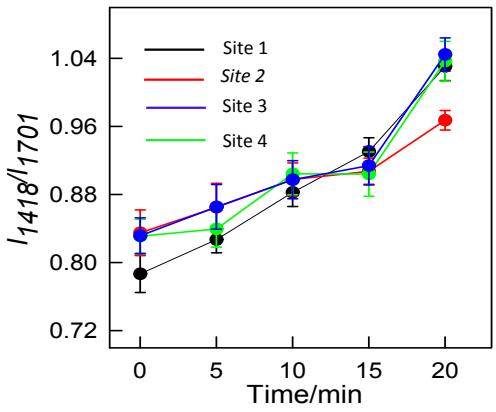

Figure S12. Temporal evolutions of the intensity ratio of I_{1418}/I_{1701} in the different sites during the PTT.

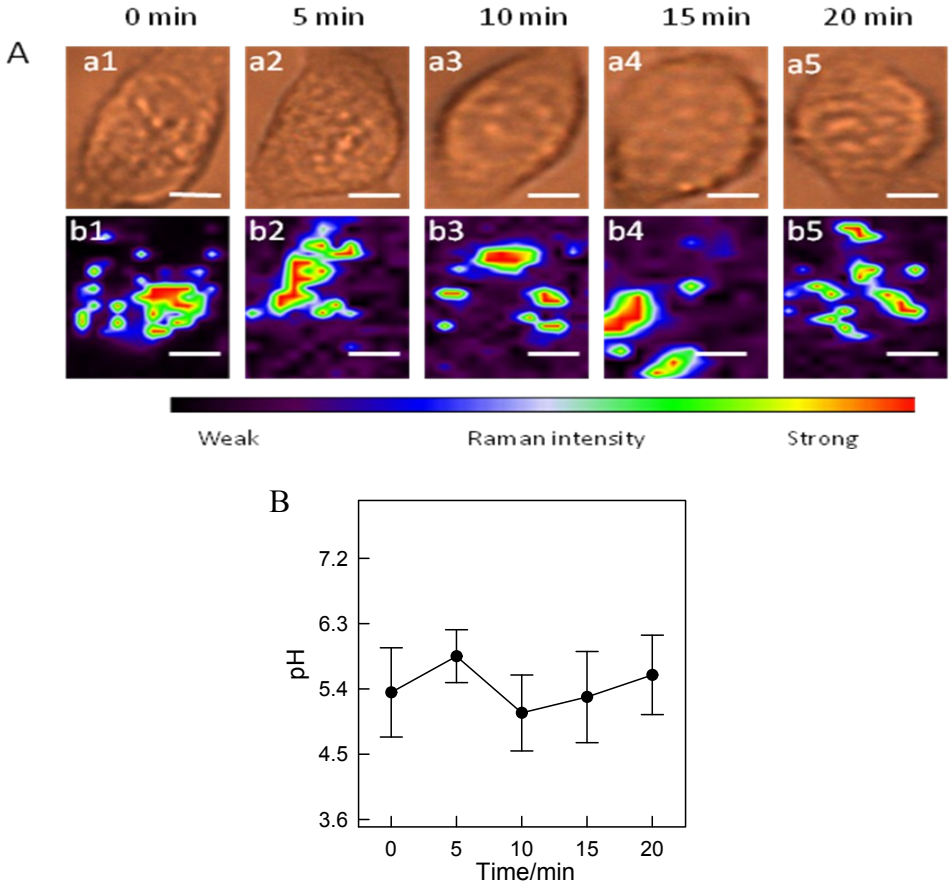

Figure S13. (A) SERS imaging to monitor the dynamic change of local pH in individual HeLa cell without AMP over illumination time (0, 5, 10, 15 and 20 min). (a1-a5) represent bright-fielded optical images and SERS imaging (b1-b5) of HeLa cells, respectively. Scale bar: 5 μ m . (B) Plot of pH in lysosome as a function of illuminated for different time.

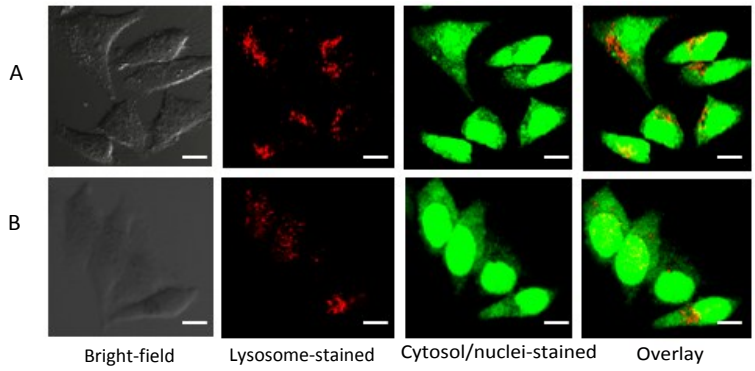

Figure S14. AO stained images of HEPES-treated (A) and AMP-treated (B) HeLa cells without illumination**.** [AMP]=0.4 nM. Scale bar: 10 μm.

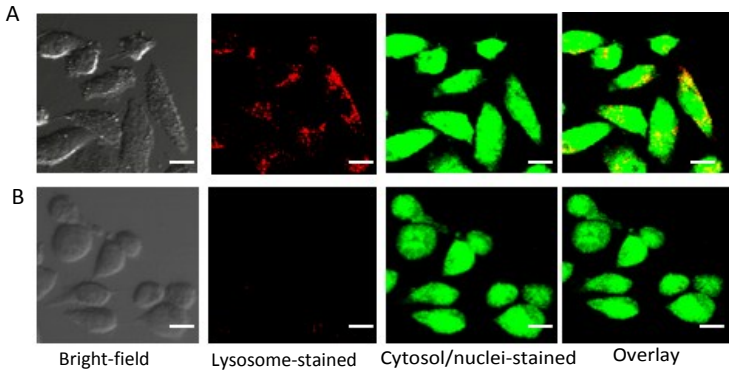

Figure S15. AO stained images of HEPES-treated (A) and AMP-treated (B) HeLa cells with illumination (20 min). [AMP]=0.4 nM.

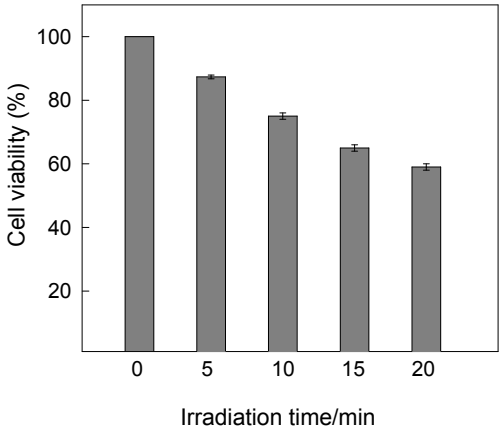

Figure S16. Cell relative viability of AMP-incubated HeLa cells treated with 780 nm CW laser irradiation at the dose of 2.0 W/cm^2 for different duration time. [AMP]=0.4 nM.