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Supporting Information

Mitochondria-targeting Au Nanoclusters Enhanced Radiosensitivity of Cancer Cells

Xu Fang,[†] Yaling Wang,[‡] Xiaochuan Ma,*,[§] Yingying Li,[†] Zhaolei Zhang,[†] Zhisheng Xiao,[†] Lijia Liu,[†] Xueyun Gao,[‡] Jian Liu*,[†]

† Institute of Functional Nano and Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Soochow University, Suzhou, Jiangsu 215123, China

‡ Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China

§ School for Radiological and Interdisciplinary Sciences (RAD-X), Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Soochow University, Suzhou, Jiangsu 215123, China

Correspondence to: jliu@suda.edu.cn; xcma@suda.edu.cn

Supplementary Experimental Procedures

Photostability comparison of AuNCs, Mito-Green and Mito-Red

The solution containing 1×10^5 cells/mL (MCF7) was plated in 35 mm glass bottomed culture dishes for cell culture at 37 ^oC under an atmosphere containing 5% CO_2. Two types of dyes (Mito-Green and Mito-Red) for mitochondria staining were chosen as reference agents. In the photostability comparison experiment, CCYKFR-AuNCs were incubated with cells for 4 h. The commercial dyes were incubated with cells for 15 min by referring to the manufacturer's protocol (200 nM Mito-Green or 100 nM Mito-Red respectively). The three samples were continuously irradiated by 405 nm laser for different time intervals in 40 min. In order to guarantee reliable comparison, the setting-up parameters on the confocal laser scanning microscope (TCS SP5, Leica) were kept identical. The fluorescence of the cell samples was monitored, and then normalize by their initial intensities for calculation of change percentages.

The absolute quantum yield can be calculated by referring to the standard method [L. Porrès, et al., J. Fluorescence, 16, 2006].

Co-localization analysis of the CCYKFR-AuNCs on mitochondria

The co-localization analysis of the fluorescent images was performed by an open source software (Image J) with the IntraCell plugin. Based on an algorithm for pixel selection and pixel-to-pixel intensity analysis, localization of AuNCs and Mito-Green was determined for their distribution inside the cells simultaneously. Multiple (n = 3) regions of interest (ROI) were analyzed with the identical settings. Statistical data analysis was performed using the software of Origin.

Frozen sections of MCF7 cells after the incubation with CCYKFR-AuNCs or the blank control cells were prepared by referring to a standard protocol. The images of the slices were acquired and analyzed by HAADF-STEM (Philips CM 200, TEM).

Cell viability assays

A standard method of methyl thiazolyl tetrazolium (MTT, Sigma-Aldrich) assay was used to evaluate the cytotoxicity of different types of AuNCs. MCF7 cells were seeded in the 96-well plate and grown for 24 h. They were incubated with various concentrations of CCYKFR-AuNCs (0, 20, 40, 50, 60, 70, 80 mg/mL) for 24 h or 48 h at 37 ^oC under an atmosphere containing 5% CO_2. The untreated cells were also tested by the MTT assays as a blank control for normalization of cell viability. The cytotoxicity of CCY-AuNCs were evaluated with the same method.

After MCF7 cells were incubated with CCYKFR-AuNCs and CCY-AuNCs, the cells were collected and eliminated by aqua regia. The cell uptake amounts of the Au element were quantified by ICP-MS (ELEMENT 2, Thermo).

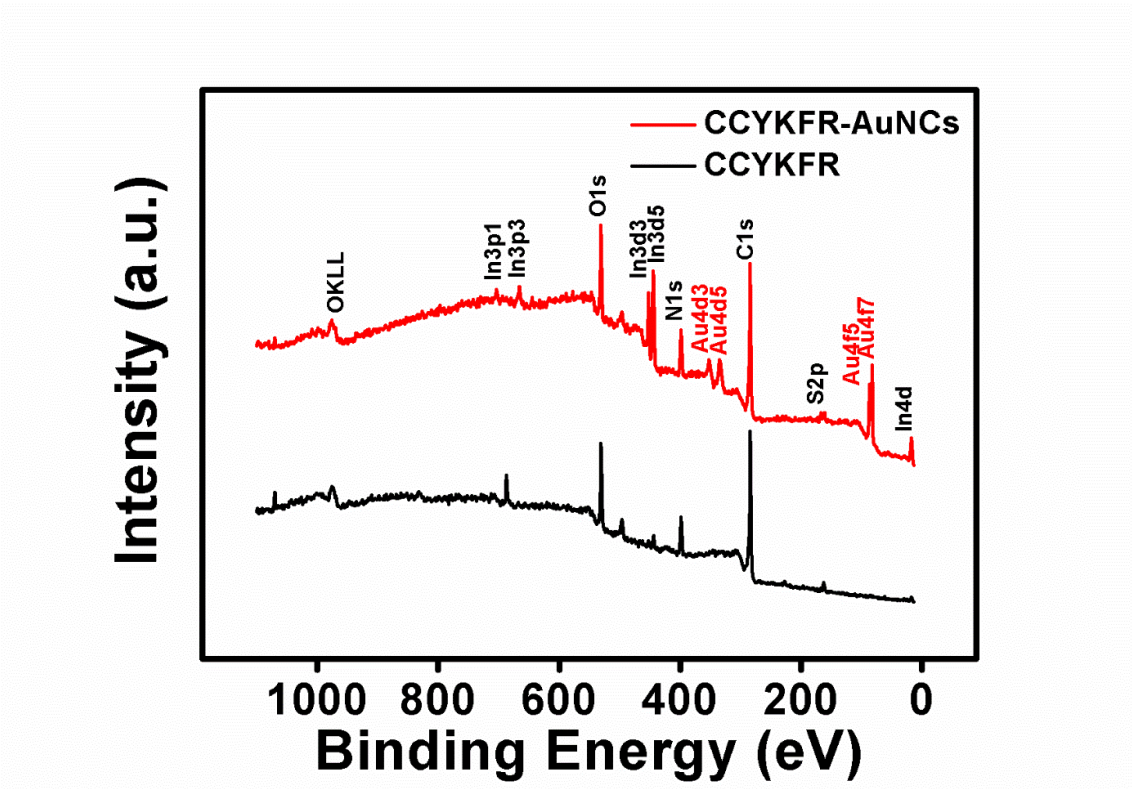

Fig. S1 XPS spectra of CCYKFR-AuNCs and CCYKFR peptide. The characteristic peaks corresponding to Au element were highlighted in the red.

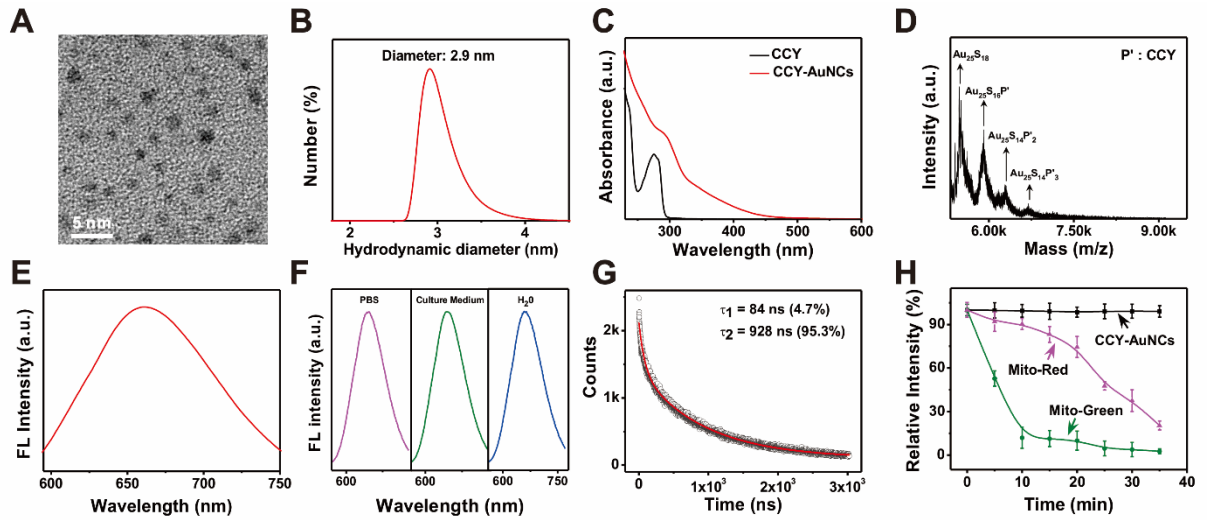

Fig. S2 Characterization of CCY-AuNCs. (A) TEM image of CCY-AuNCs. Scale bar: 5 nm. (B) Size distribution of CCY-AuNCs in cell culture medium. (C) Absorption spectra of CCY-AuNCs and CCY peptide. (D) MALDI-TOF MS of CCY-AuNCs in the linear mode, using CHCA as the matrix. (E) Spectra of emission fluorescence of CCY-AuNCs. (F) Fluorescent spectra of CCY-AuNCs dispersed in deionized water, PBS, or RPMI-1640 cell culture medium. (G) Fluorescence lifetime of CCY-AuNCs. (H) Photostability of CCY-AuNCs, in comparison with Mito-tracker Green (Mito-Green) and Mito-tracker Red (Mito-Red). Error bar: standard deviation (n=3).

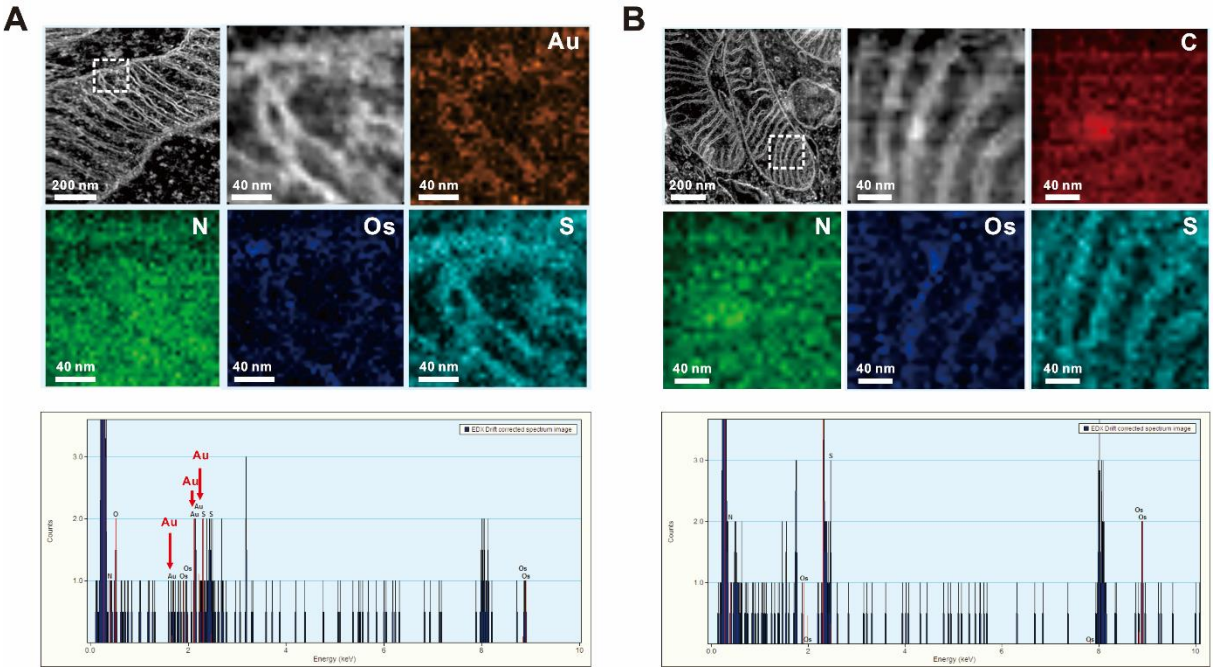

Fig. S3 HAADF-STEM images of mitochondria from the sectioned cell samples. (A) MCF7 cells treated with CCYKFR-AuNCs. (B) MCF7 cells without treatment as the blank control. The square highlighted by the dash line in the first panel is magnified for the subsequent zoomed-in view and analysis. Au element mapping on the mitochondria is shown in the orange.

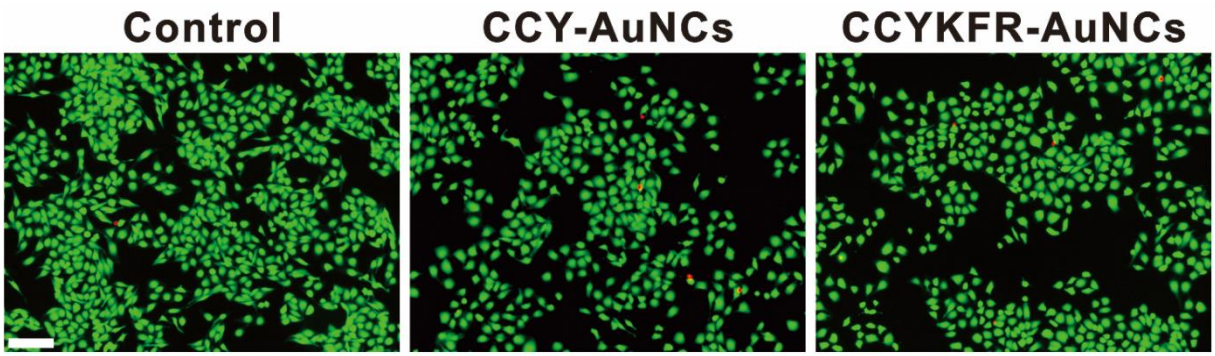

Fig. S4 AM (green, showing living cells) and PI (red, showing dead cells) dual-color staining of MCF7 cells treated with CCY-AuNCs or CCYKFR-AuNCs. Non-treated cells were stained in parallel as the blank control. Scale bar : 75 μm.

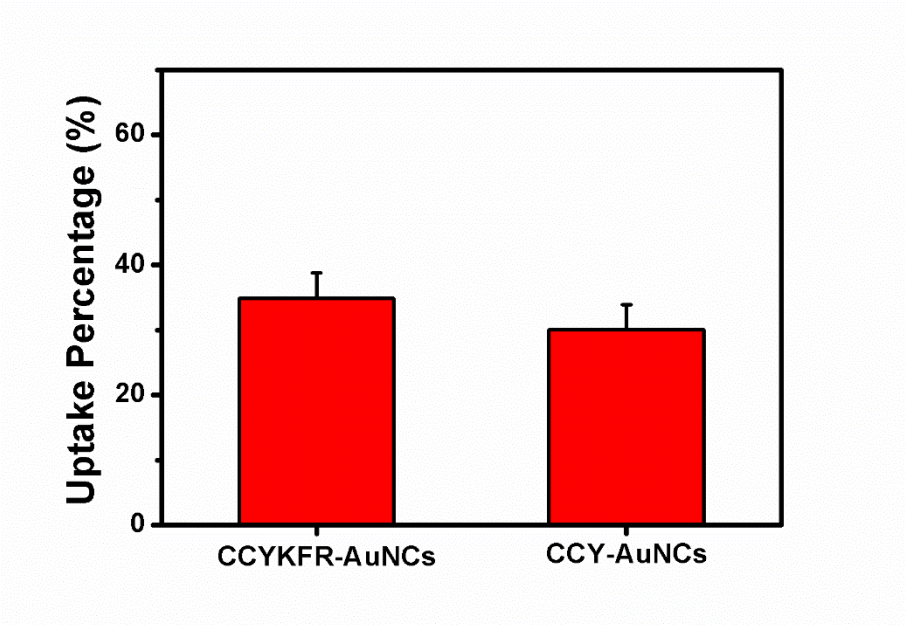

Fig. S5 Cellular uptake of Au element by ICP-MS. MCF7 cells were incubated with CCYKFR-AuNCs (40 μg/mL) and CCY-AuNCs (40 μg/mL) respectively. Then the cells were washed by PBS for 3 times before the measurements of ICP-MS.

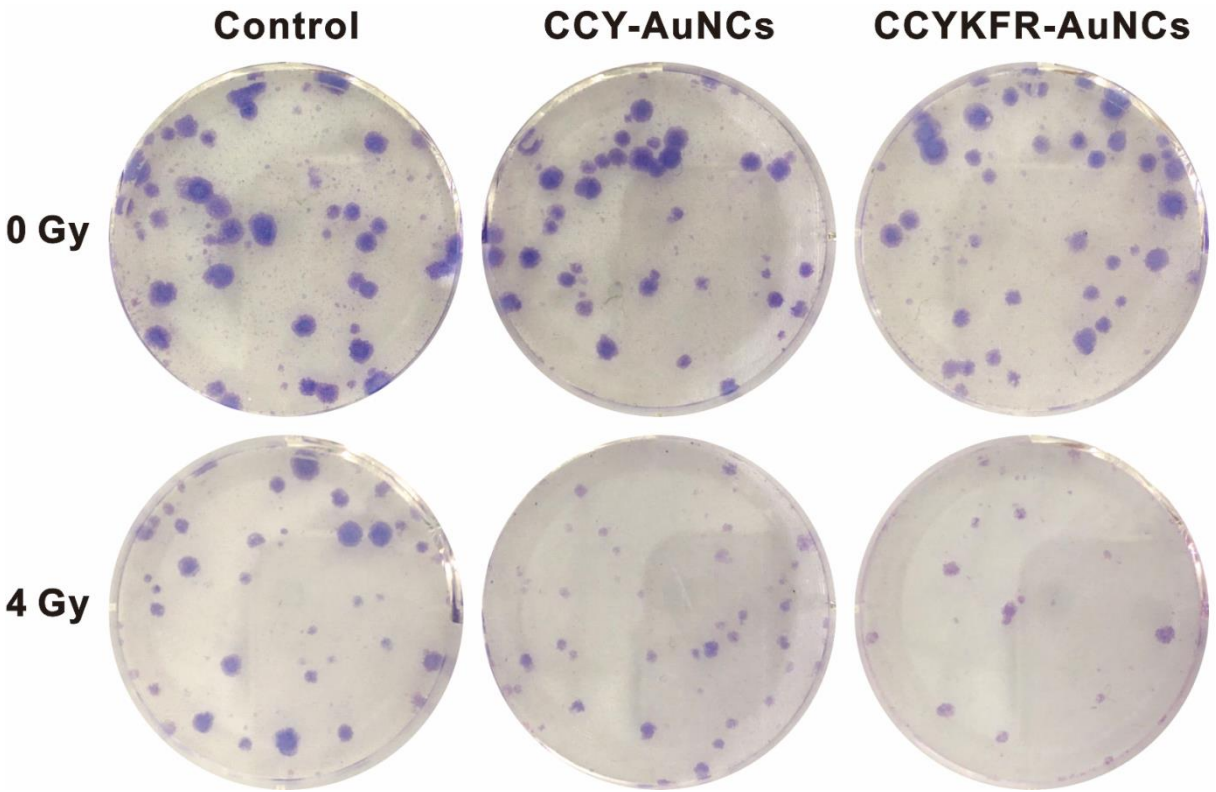

Fig. S6 Growth of MCF7 cell colonies after X-ray irradiation (4 Gy) in the clonogenic assays for 10 days..

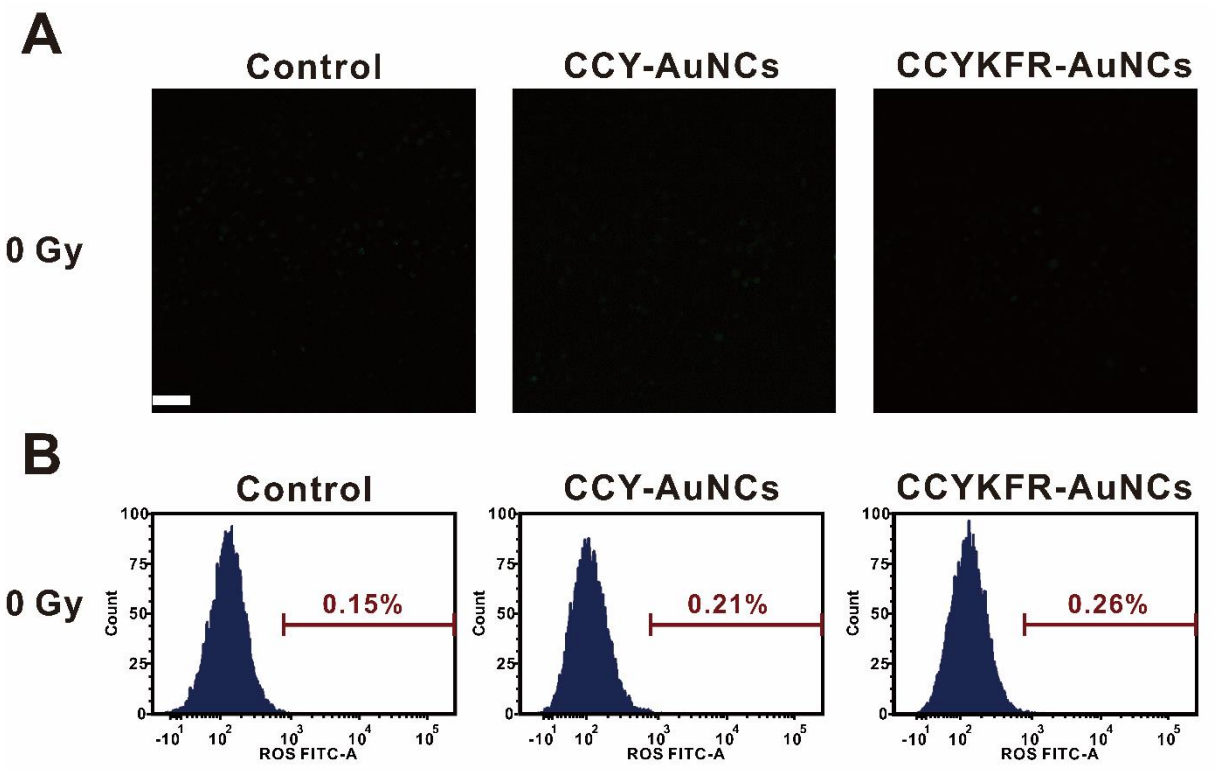

Fig. **S7** Intracellular ROS measurements in the radiosensitization experiments (0 Gy). (A) Fluorescence images showing the intracellular ROS levels of MCF7 cells in different peptide-AuNCs treatments (the untreated cells as blank control). Scale bar: 75 μm. (B) Quantitative data of the ROS level by flow cytometry analysis.