## Autophagy-inhibiting biomimetic nanodrugs enhance photothermal therapy and boost antitumor immunity

Pei-Ying Huang<sup>a</sup>, Yin-Yin, Zhu<sup>a</sup>, Hao Zhong<sup>a</sup>, Pei-Ling Chen<sup>a</sup>, Qun-Ying Shi<sup>a</sup>, Jiao-Yu Chen<sup>a</sup>, Jin-Mei Lai<sup>a</sup>, Ying-Feng Tu<sup>\*a</sup>, Shu-Wen Liu<sup>\*ab</sup>, and Li-Han Liu<sup>\*a</sup>

<sup>a</sup> Guangdong Key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, Guangdong, P. R. China

<sup>b</sup> State Key Laboratory of Organ Failure Research, Guangdong Provincial Institute of Nephrology, Southern Medical University, Guangzhou 510515, China

## \* Corresponding E-mail address:

tuyingfeng1@smu.edu.cn (Ying-Feng Tu) liusw@smu.edu.cn (Shu-Wen Liu) liulihan@smu.edu.cn (Li-Han Liu)



**Fig. S1.** (A) Temperature elevation of 4T1 cells with ICG of NIR laser in 96-well plate (ICG: 10  $\mu$ M, photodensity: 1.5 W cm<sup>-2</sup>) (n = 3). (B) Representative immunofluorescence images of LC3 punctate dots after treating with ICG of NIR laser (ICG: 10  $\mu$ M, photodensity: 1.5 W cm<sup>-2</sup>). Scale bar: 50  $\mu$ m. (C) Western blot assay of Casp-3 expression in 4T1 cells after different treatments (ICG: 10  $\mu$ M, photodensity: 1.5 W cm<sup>-2</sup>). MFI means Mean fluorescence intensity.



**Fig. S2.** (A) Hydrodynamic diameter and (B) the surface zeta potential values of ICGCQ. (C) The representative TEM image of ICGCQ NPs (Scale bar = 500 nm). (D) The stability of ICGCQ in PBS and 1640 medium.



**Fig. S3.** (A) The representative TEM image of ICGCQ@RCm NPs. (B) The hydrodynamic size changes of ICGCQ@RCm NPs in PBS for 5 days.



**Fig. S4**. Linear time data versus  $-\ln(\theta)$  of ICGCQ@RCms NPs (A) and ICG (B) obtained from the cooling period of NIR laser off. (C) Fluorescence intensities of ICGCQ@RCms NPs and ICG dispersions in water after laser irradiation at different times. MFI is an abbreviation for mean fluorescence intensity.



Fig. S5. CLSM images of 4T1 cells after treatment with ICGCQ@RCms NPs for 2, 4, and 8 h, respectively (ICG: 10  $\mu$ M). Scale bar: 40  $\mu$ m. The numbers inserted in the upper left corners are the semiquantitative mean fluorescence intensity of ICG.



Fig. S6. Representative mean fluorescence intensities for 4T1 cells after treatment with ICGCQ@RCms NPs for 2, 4, and 8 h, respectively (ICG:  $10 \mu$ M).



Fig. S7. Mean fluorescence intensities of 4 hours incubation with free ICG, ICGCQ NPs, ICGCQ@RCms NPs (ICG:  $10 \mu$ M).



Fig. S8. Mean fluorescence intensities of the five cell lines 4T1, MCF-7, 3T3, CT-26 and Raw 264.7 upon 4 h incubation with ICGCQ@RCms NPs (ICG:  $10 \mu$ M).



Fig. S9. Autophagy inhibition induced by ICGCQ@RCms NPs. Representative immunofluorescence images of LC3 punctate dots after various treatments. (photodensity:  $1.5 \text{ W cm}^{-2}$ , 60 s). Scale bar:  $50 \mu \text{m}$ . The numbers inserted in the upper left corners are the semiquantitative mean intensity of green fluorescence.



Fig. S10. Preliminary biosafety assessment of ICGCQ@RCms NPs.



Fig. S11. Hematoxylin-eosin staining of the tissues (heart, liver, spleen, lung, kidney and tumor) after different treatments for assessment of toxicity (scale bars:  $10 \mu m$ ).



**Fig. S12.** Hematological parameters of the mice on the 1<sup>st</sup> day and 7<sup>th</sup> day (n = 2).