Supplementary information

Cancer Immunotherapy Based on Carbon-Quantum-Dot Modified Cancer Cells

Tao Liang#，Josh Haipeng Lei#, Jinsong Tao#, Sen Guo, Hanlu Gao, Lipeng Zhu, Yinning Zhou, You-Cheng Liu, Long Xi, Haibo Tong, Enshan Liu, Bohan Zhang, Heng Sun, Huiqi Zhang, Kathy Qian Luo, Qi Zhao, Tzu-Ming Liu, Defang Ouyang, Wei Ge, Ying Zheng*, Chu-Xia Deng*, Zikang Tang*, Songnan Qu*

* Corresponding authors, emails: songnanqu@um.edu.mo; zktang@um.edu.mo; cxdeng@um.edu.mo; yzheng@um.edu.mo

# These authors contributed equally to this work.

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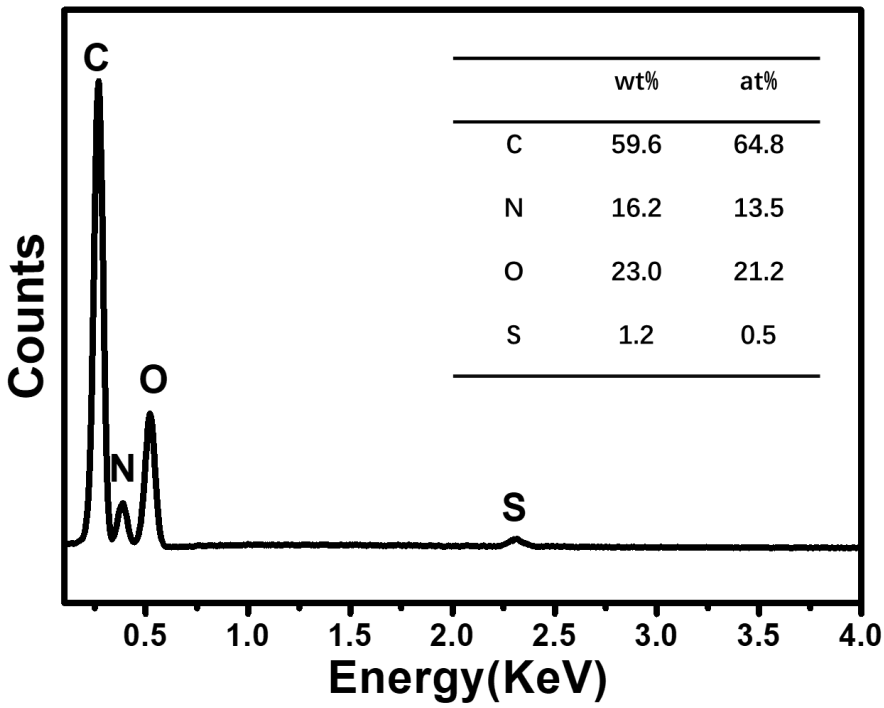
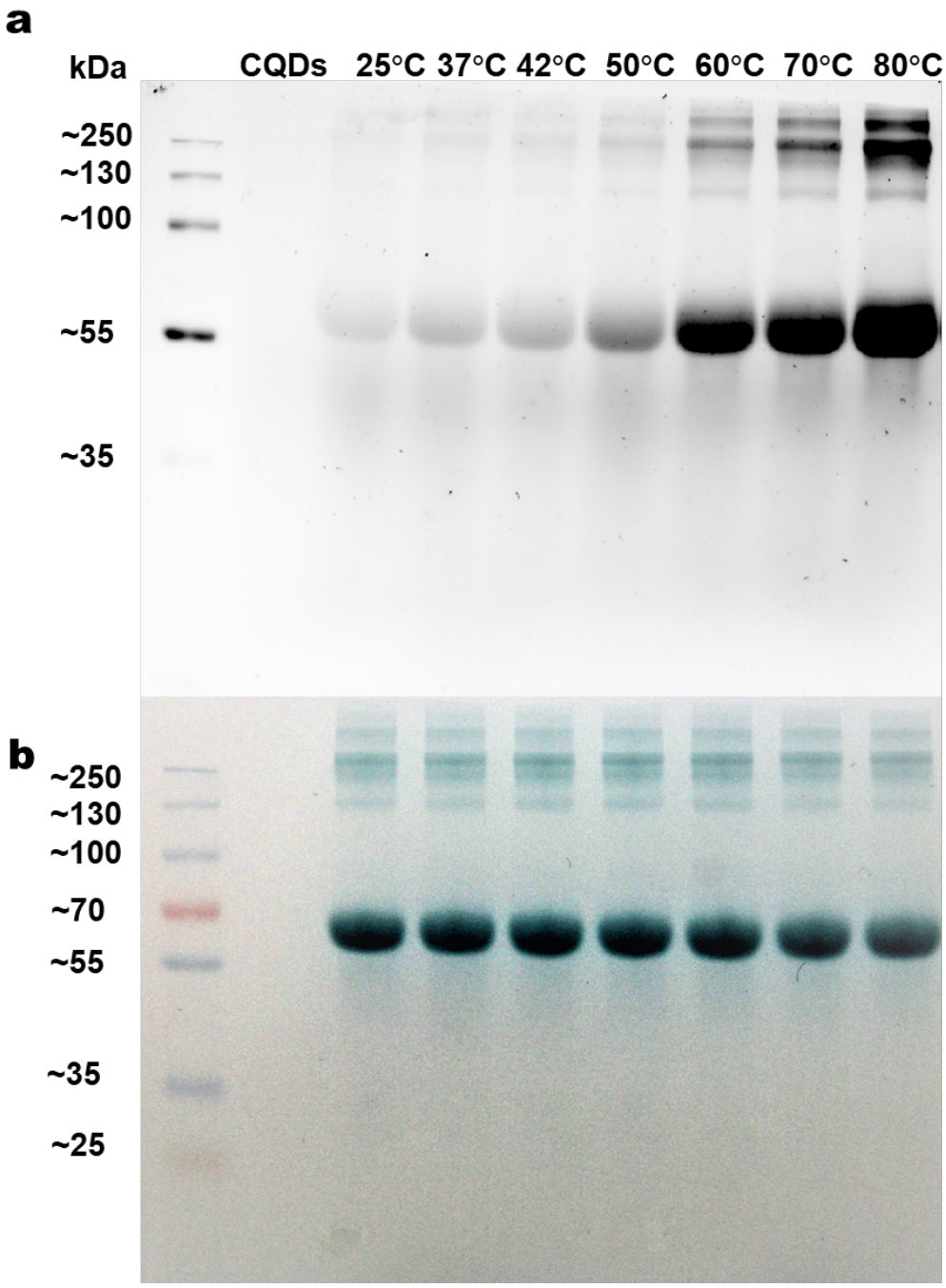
Figs. S1 to S21

Tables S1-S2

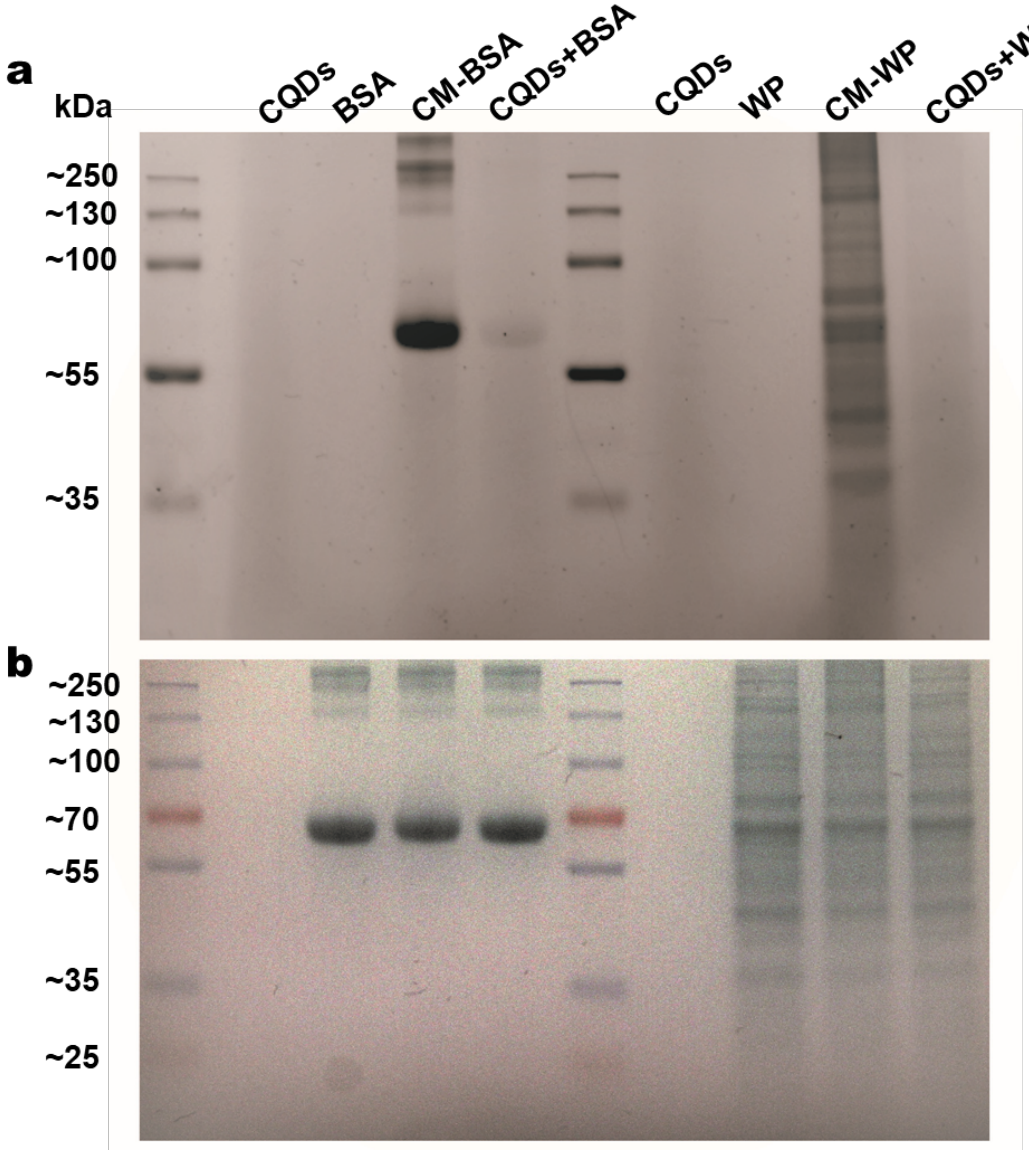


	wt%	at%
C	59.6	64.8
N	16.2	13.5
O	23.0	21.2
S	1.2	0.5

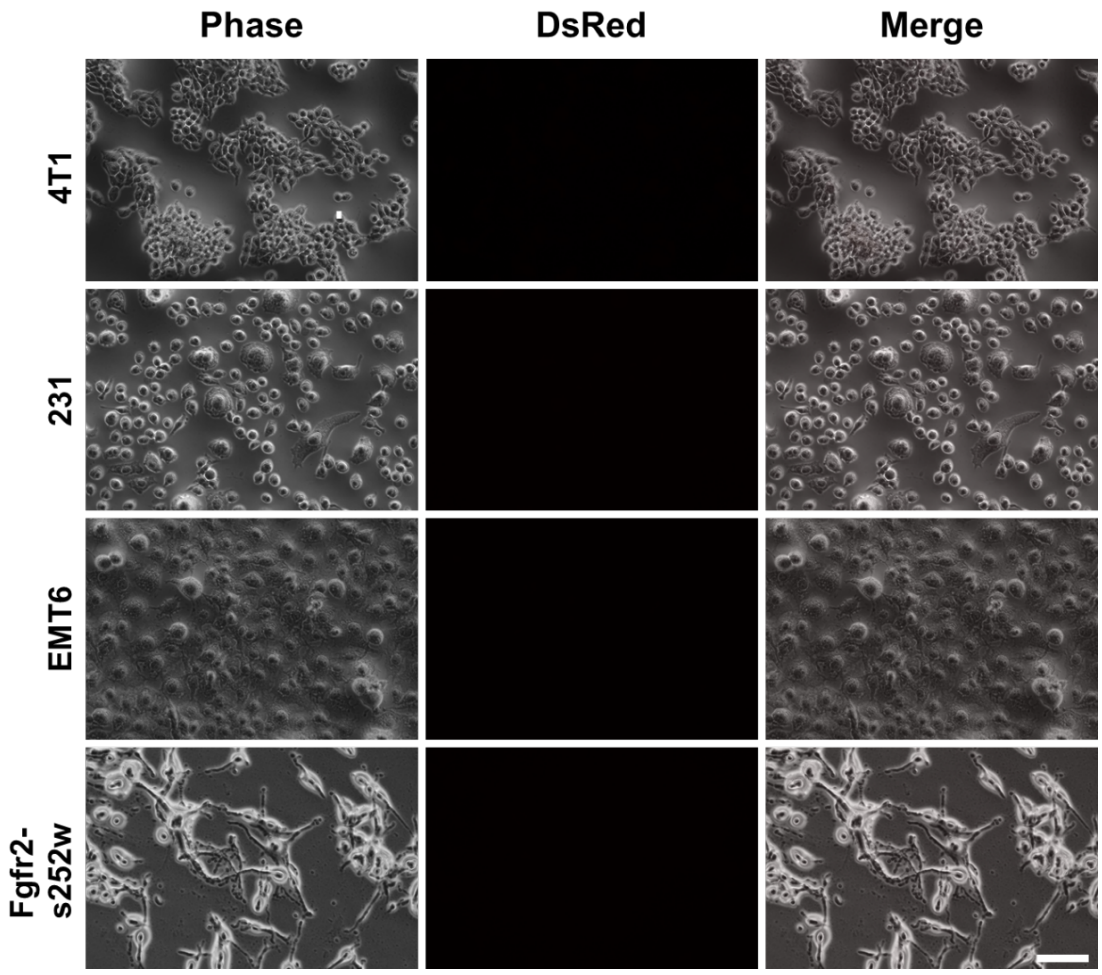
Supplementary Fig. 1. EDS survey spectrum of CQDs. The presence of C, O, N, and S elements are with weight contents of 59.6, 23.0, 16.2 and 1.2 %, respectively.



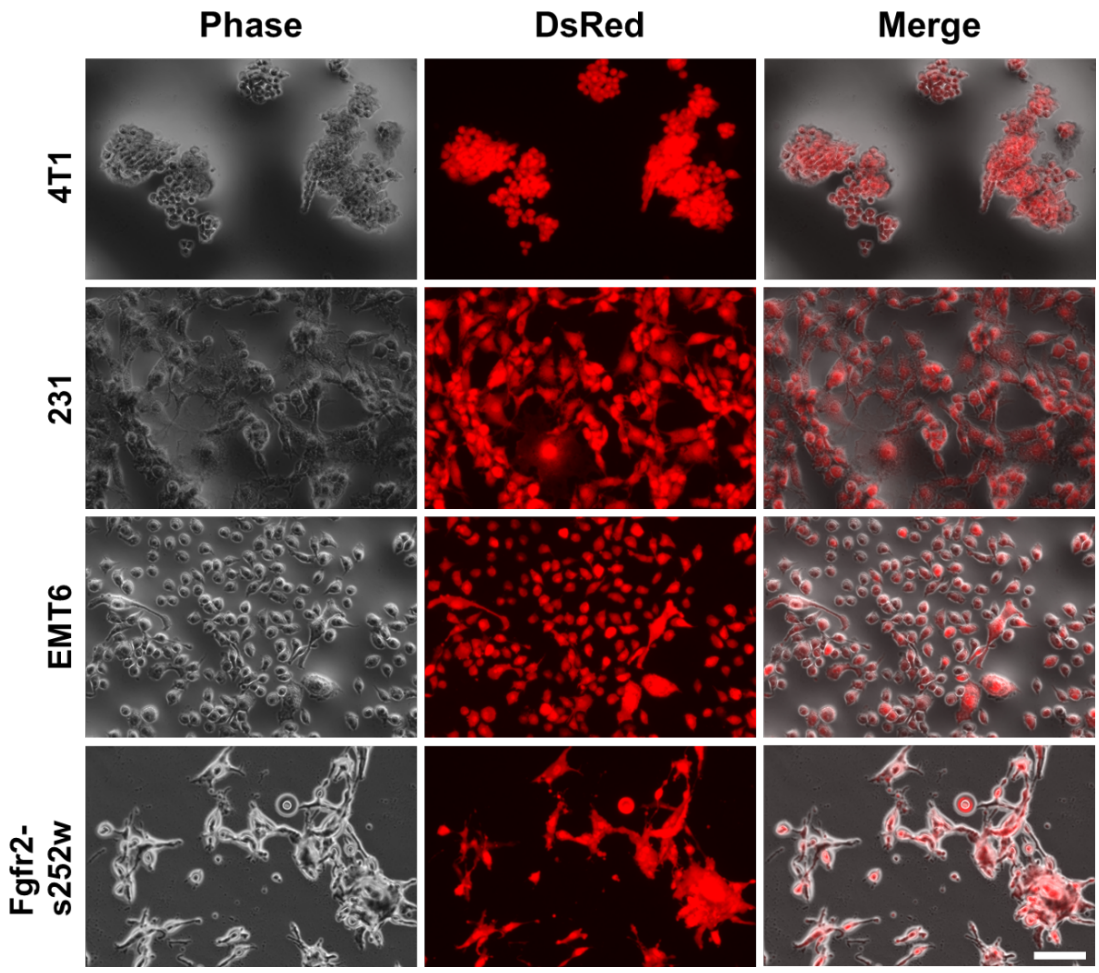
Supplementary Fig. 2. SDS-PAGE patterns of pure CQDs, and BSA with CQDs after different temperature annealing. (a) Fluorescence bands (excitation: 647 nm, filter: 710 nm); **(b)** Coomassie Brilliant Blue staining bands. From left to right: CQDs, BSA with CQDs after annealed at 25°C, 37°C, 42°C, 50°C, 60°C, 70°C, 80°C.



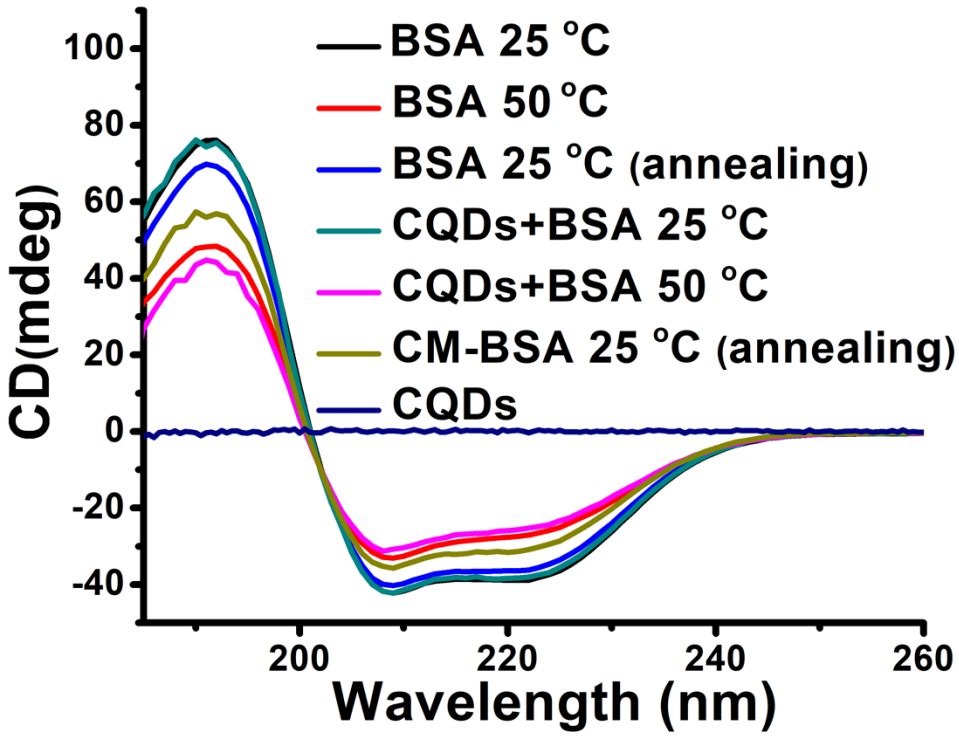
Supplementary Fig. 3. SDS-PAGE patterns of CQDs, BSA, CM-BSA, CQDs+BSA, WP, CM-WP and CQDs+WP. (a) Flurenscence bands (excitation: 647 nm, filter: 710 nm); **(b)** Coomassie Brilliant Blue staining bands.



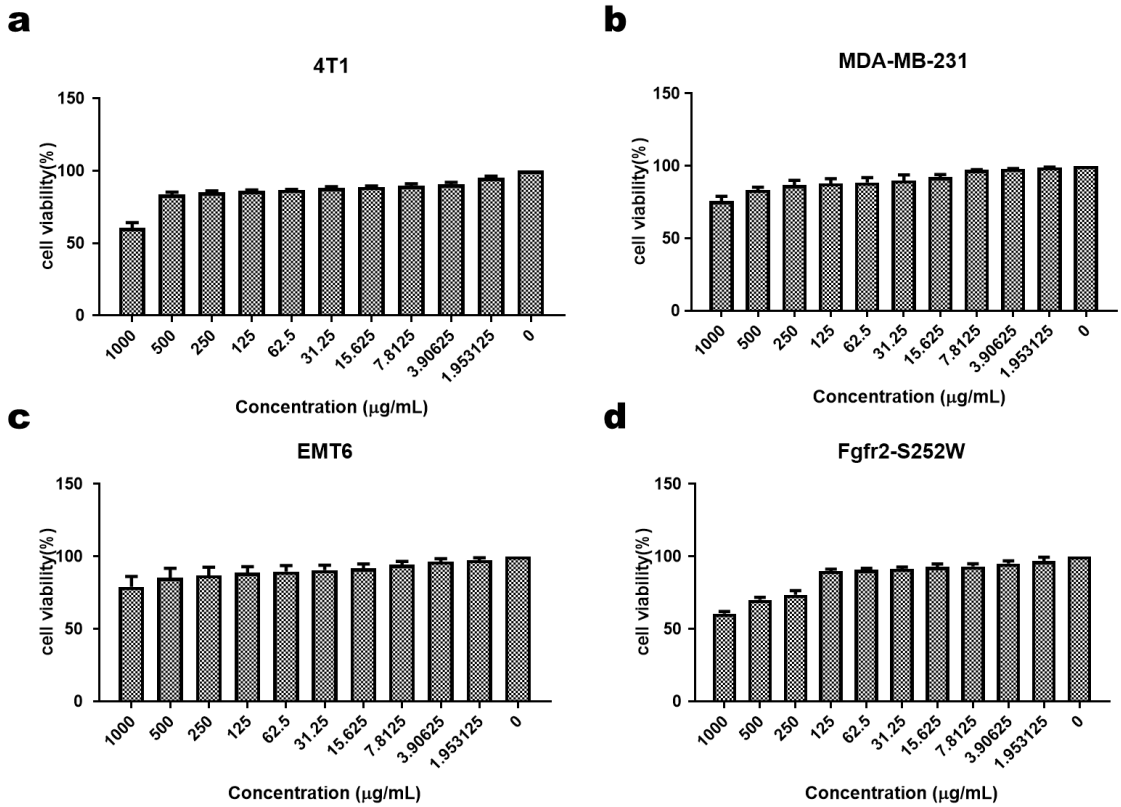
Supplementary Fig. 4. Optical and fluorescence microscopy images of different cells groups co-incubated with CQDs for 24 hr. CQDs cannot be actively ingested by living cells or bonded to the cell surfaces at body temperature. Cells are chosen from different cells such as 4T1, MDA-MB-231, EMT6 and tumor cells from *Fgfr2-S252W* model mice. Scale bar: 100 μm.



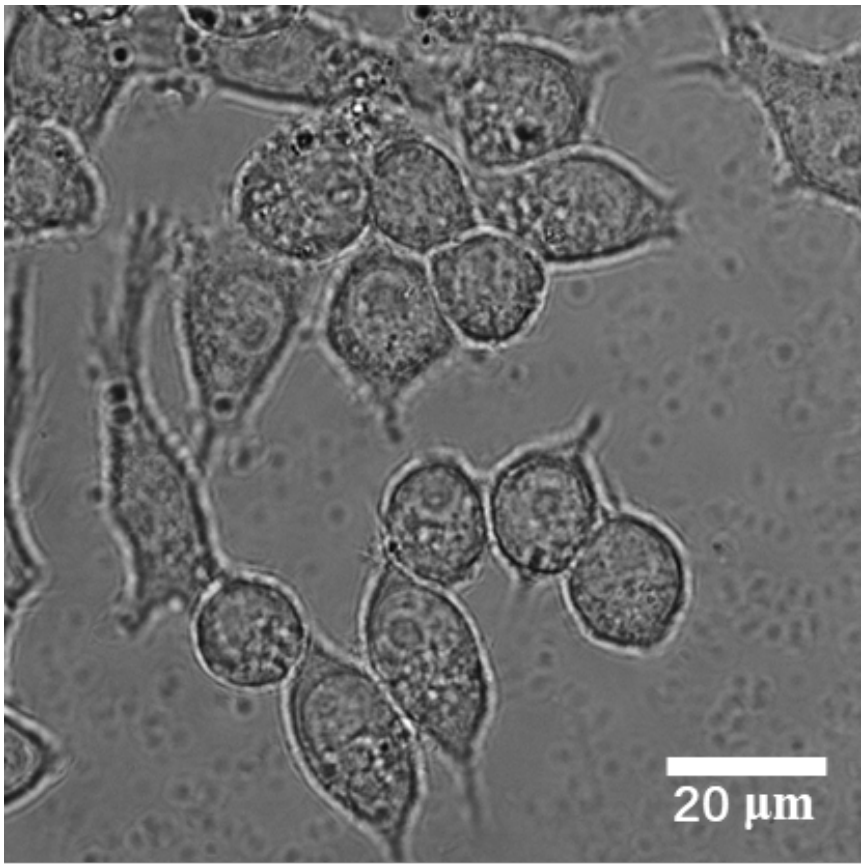
Supplementary Fig. 5. Optical and fluorescence microscopy images of different cells (4T1, MDA-MB-231, EMT6 and tumor cells from *Fgfr2-S252W* model mice) co-incubated with CQDs after annealing at 50 ºC for 10 mins. Before imaging, the stock solution of the cancer cells was replaced by PBS. Scale bar: 100 μm.



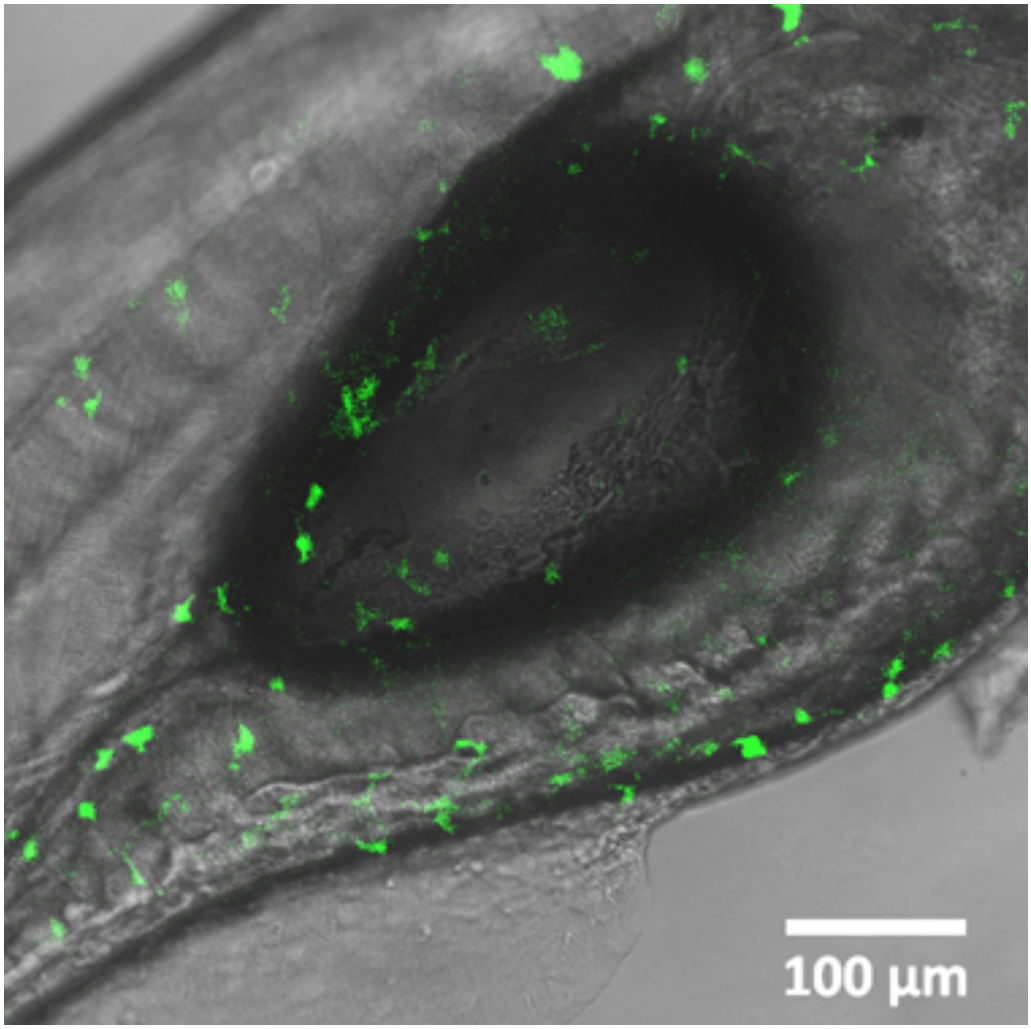
Supplementary Fig. 6. Circular dichroism spectra of CQDs, BSA and BSA+CQDs with or without thermal annealing in aqueous solutions at room temperature and BSA and BSA+CQDs in aqueous solutions at 50 ºC**.** BSA and CQDs concentrations in the solutions are 50 μg/ml and 10 μg/ml, respectively.



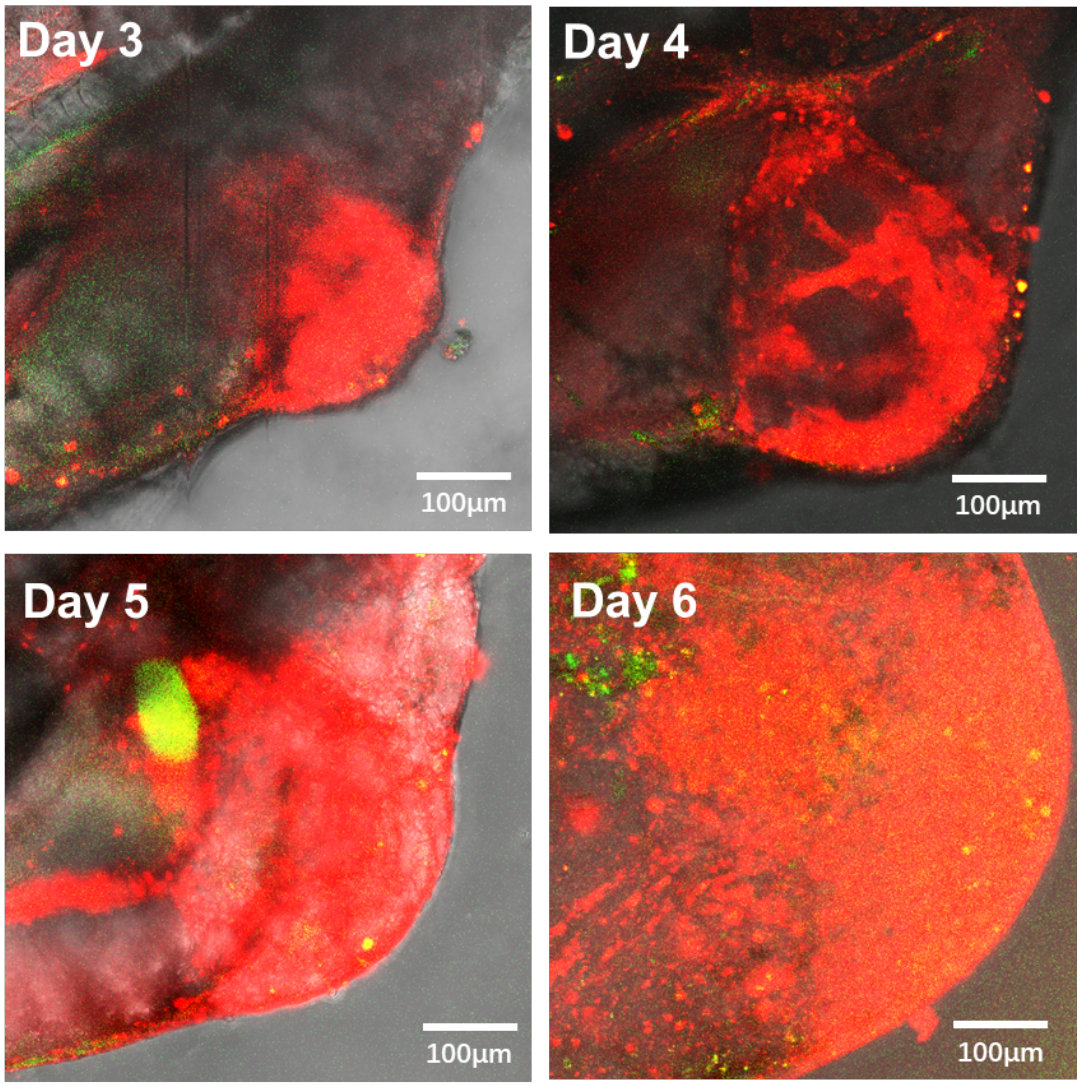
Supplementary Fig. 7. Cytotoxicity of CQDs in different cells groups. The CQDs did not affect cell viability even with concentrations up to 500 μg/ml after 48 h, indicating non- or extremely low cytotoxicity. Cells tested are (**a)** 4T1, **(b)** MDA-MB-231, **(c)** EMT6 and **(d)** *Fgfr2-S252W*.



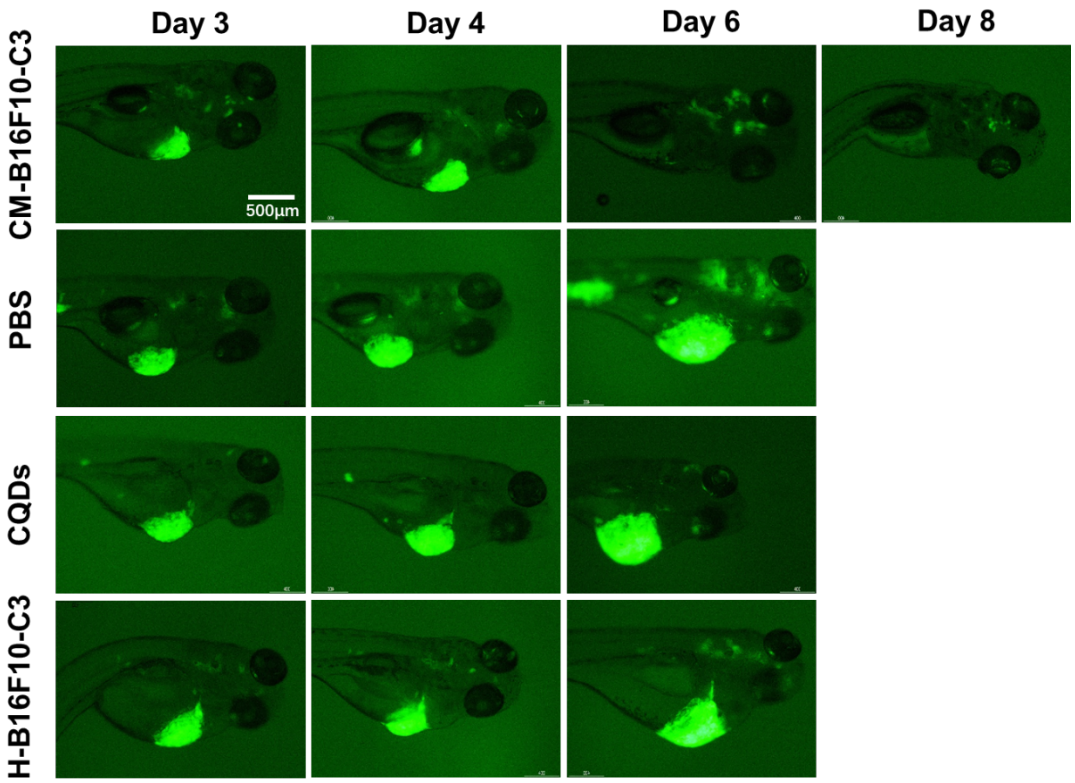
Supplementary Fig. 8. CLSM **image of RAW**264.7 **macrophage after co-incubating with PBS for 6 hr.**



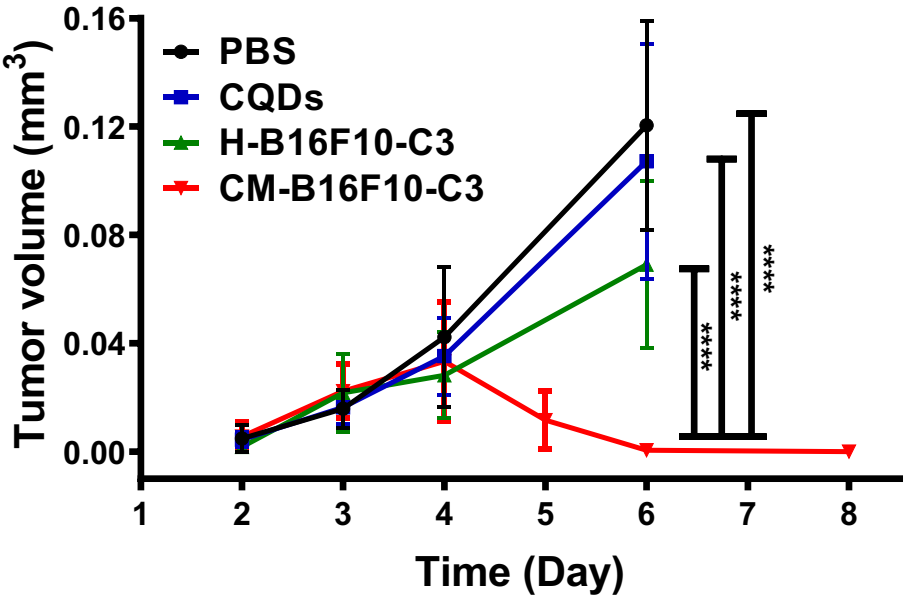
Supplementary Fig. 9. CLSM **image of macrophages in *Tg* (*mpeg1: EGFP*) zebrafish model**, which were injected abdominal with PBS at 4 nl after 6 hours. The fluorescence images were acquired at Ex 488 nm/Em 493–550 nm for EGFP signal under the independent sequence of channel with HyD detectors.



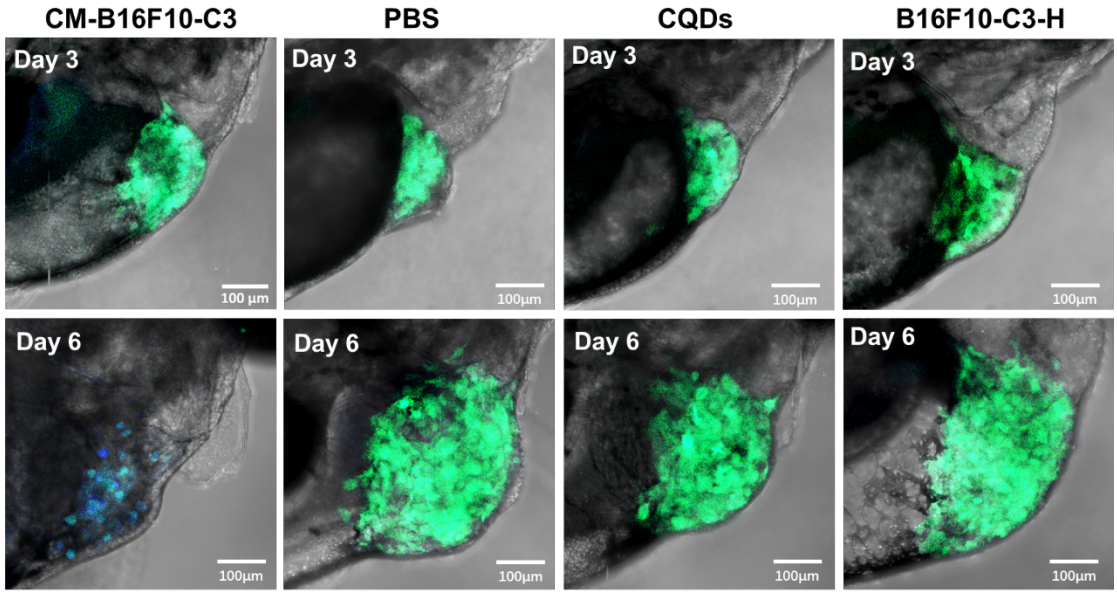
Supplementary Fig. 10. CLSM images of DiI labelled B16F10 cancer xenograft *Tg* (lck:EGFP) zebrafish model treated with PBS at 4 nl dose after different days. T cells (Green), B16F10 cells (Red). The fluorescence images were acquired at Ex 488 nm/Em 493–550 nm for EGFP signal and Ex 552 nm/Em 558–650 nm for DiI signal detection under the independent sequence of channel with HyD detectors.



Supplementary Fig. 11. Visual observation of the cancer growth by the YFP imaging under fluorescence microscope in B16F10-C3 cancer xenograft zebrafish model treated with CM–B16F10-C3, PBS, CQDs, and H-B16F10-C3 at day 3, 4, 6 and 8, respectively. CQDs: 200 μg/ml, 4 nl. B16F10-C3 cells: 36. Green fluorescence areas represent B16F10-C3 cells.



Supplementary Fig. 12. Tumor growth curves of B16F10-C3 xenograft zebrafish model in control groups (PBS, CQDs and H-B16F10-C3) and treated group (CM-B16F10-C3). Growth curves represent means ± SD. (n = 6, $p < 0.0001$).



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102 **Supplementary Fig. 13. CLSM images of monitoring the cancer cell proliferation**
103 **growth** (green fluorescence) and detect the caspase-3 activation based apoptotic (blue
104 fluorescence) by FRET imaging in B16F10-C3 cancer xenograft zebrafish model
105 before (day 3) and after (day 6) CM-B16F10-C3, PBS, CQDs and H-B16F10-C3
106 treatments, respectively.

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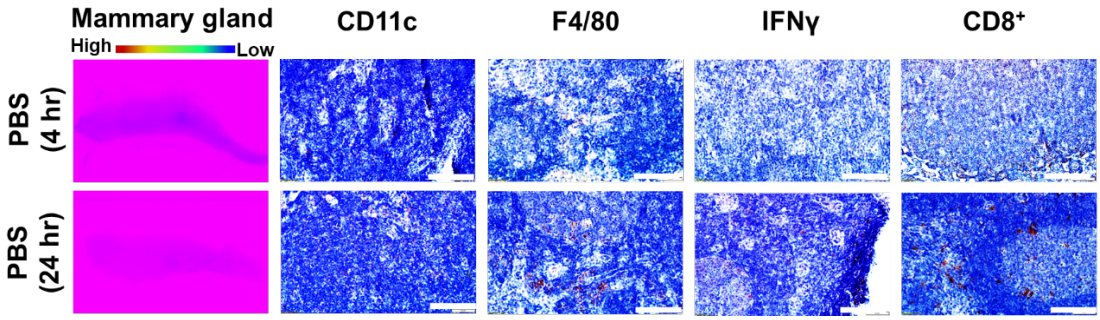
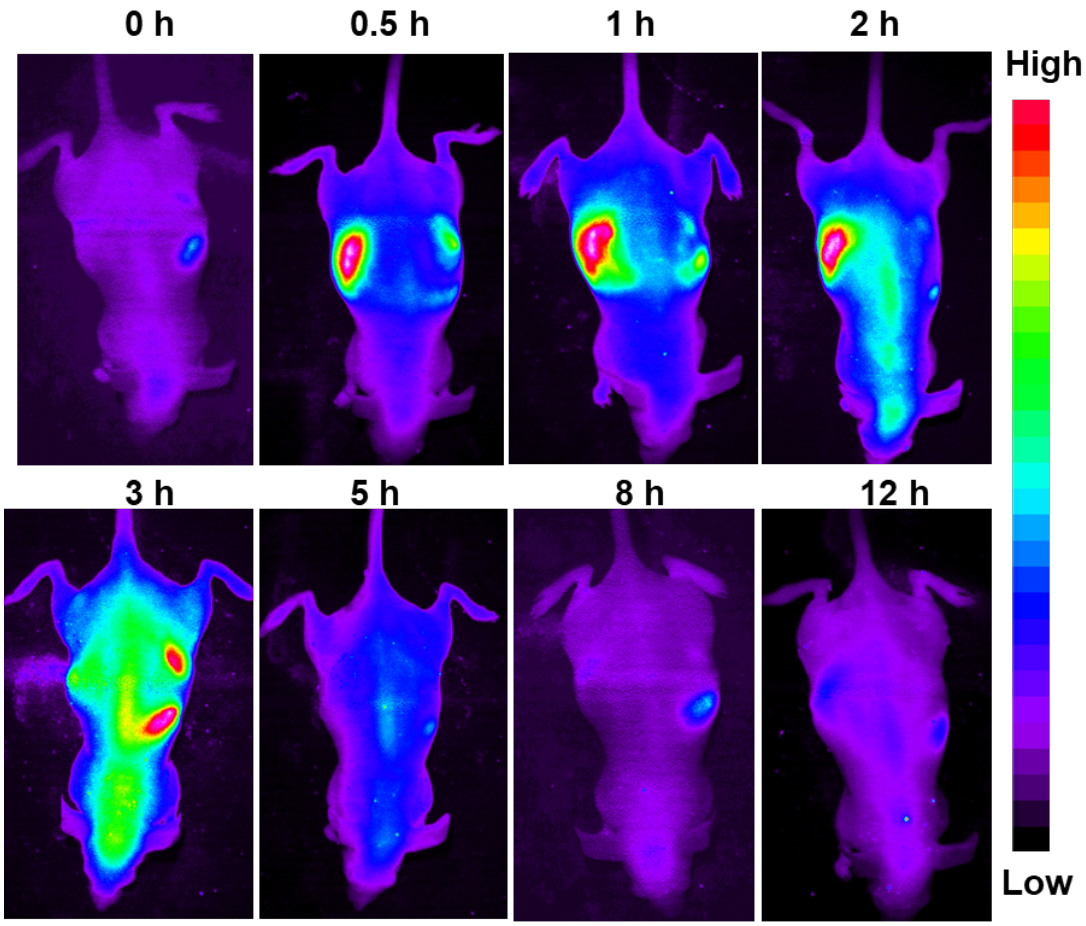
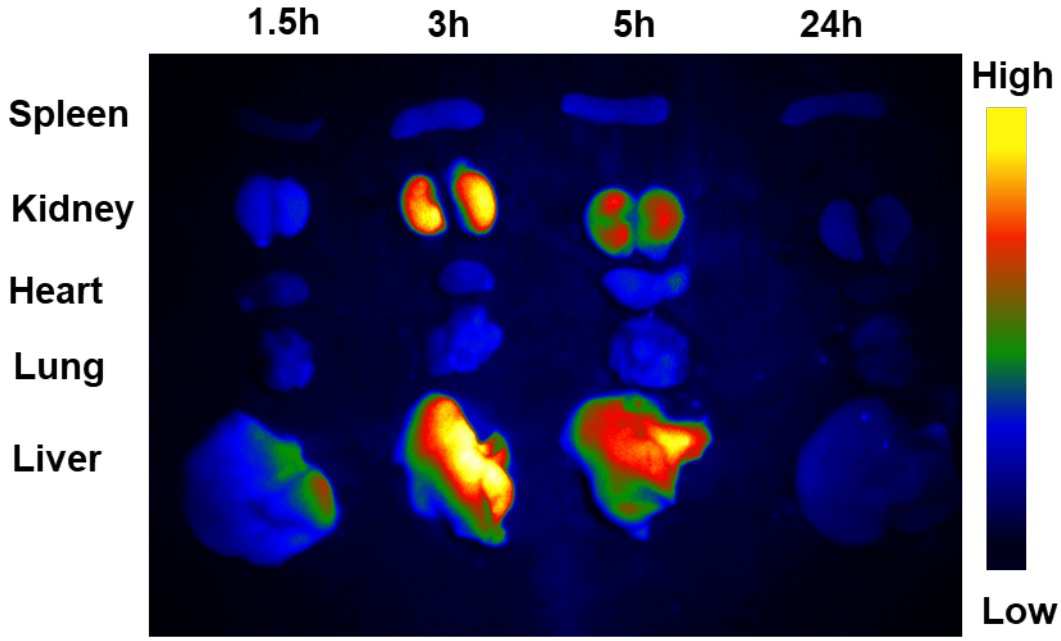


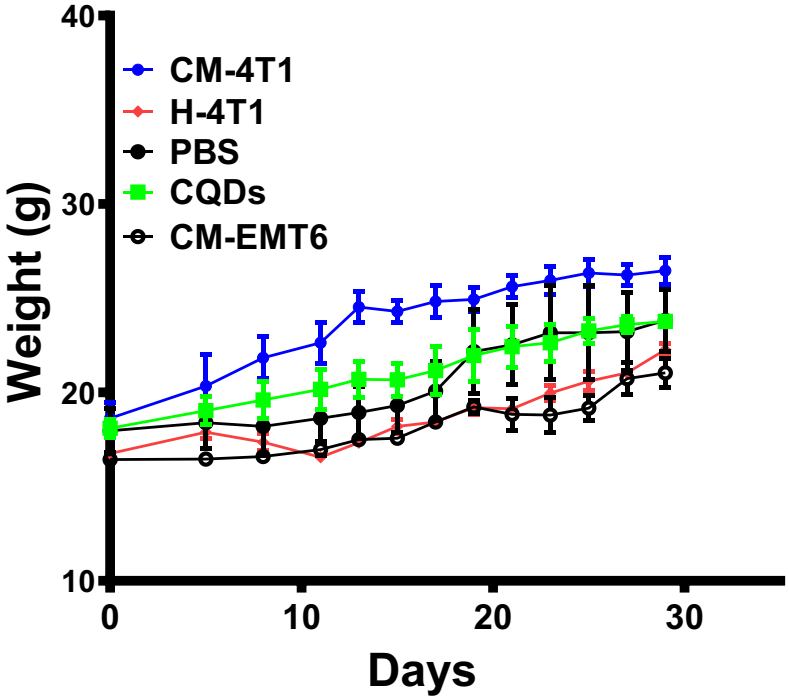
Supplementary Fig. 14. Fluorescent images of the 4th mammary tissue with inguinal lymph node (left); Balb/c mice were injected abdominal with PBS at dose of 200 µl per mice; The mammary were collected after injection for 4 hr and 24 hr (Scale bar: 3 mm). IHC staining against CD11c, F4/80 IFNγ, and $CD8^+$ with PBS treatment group (Scale bar: 100 µm).



Supplementary Fig. 15. NIR fluorescence images of a nude mouse before and after intraperitoneal injection of CM-4T1 at different time. CQDs：200 μg/ml, 100 μl .4T1 cells: 1×10^7/ml. Excitation: 655 nm. Filter: 710 nm.



Supplementary Fig. 16. NIR fluorescence images of main organs from nude mice after intraperitoneal injection at different time. CQDs：200 μg/ml, 100 μl. 4T1 cells: 1×10^7/ml. Excitation: 655 nm. Filter: 710 nm.



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130 **Supplementary Fig. 17. Weight curves of mice in P0-P4 groups** treated with CM-

131 4T1 (*n* = 6), CM-EMT6 (n = 6), H-4T1 (*n* = 6), PBS (*n* = 6) and CQDs (*n* = 6). Data

132 plotted are mean ± S.E.M.

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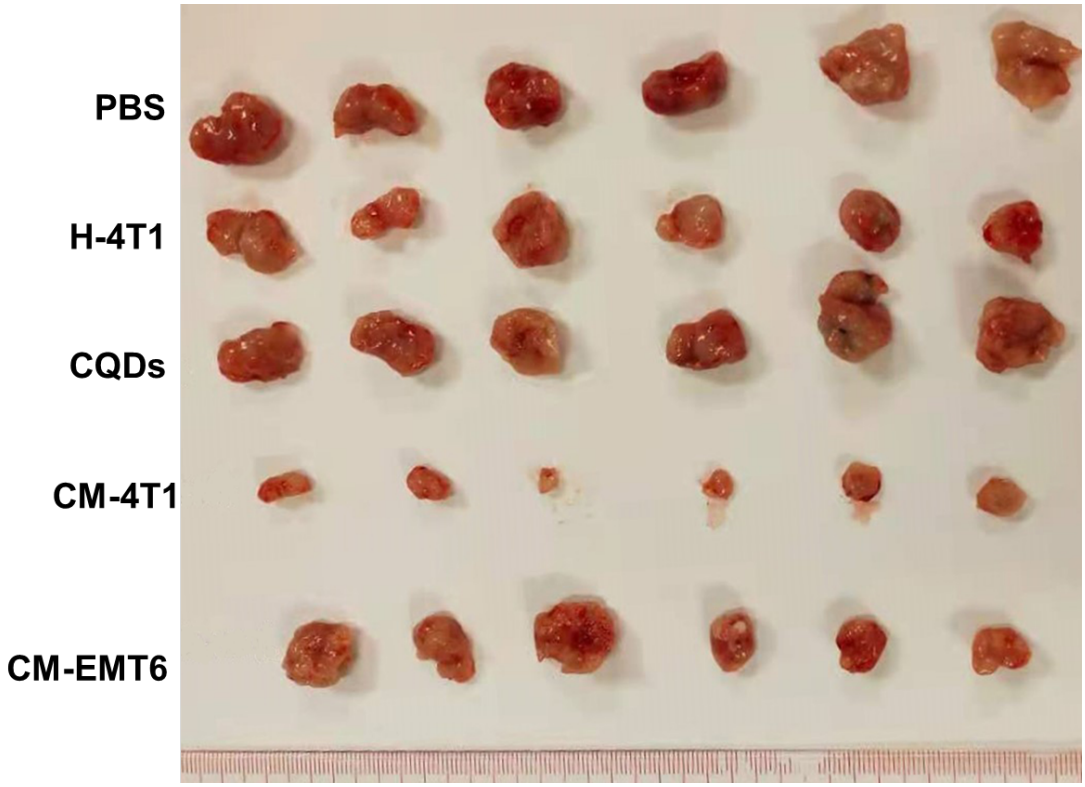
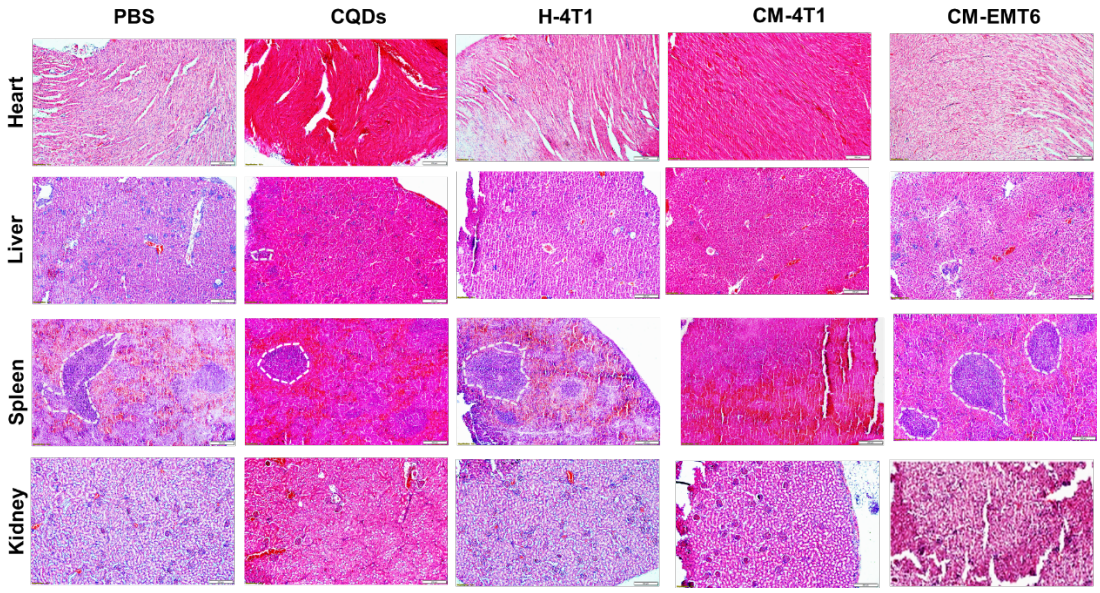
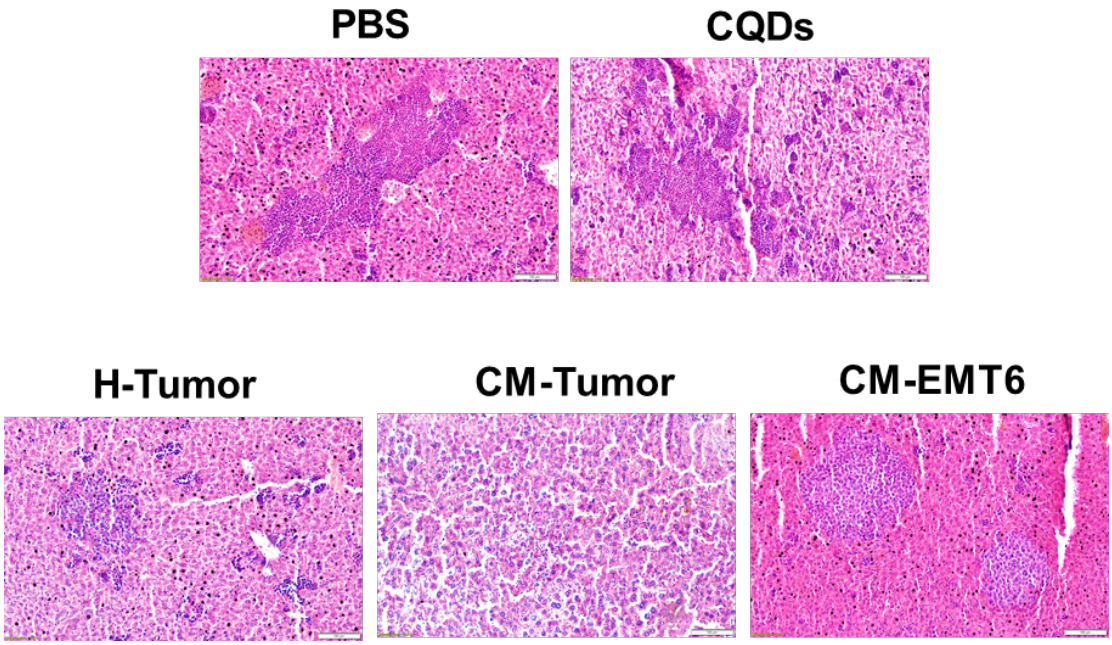


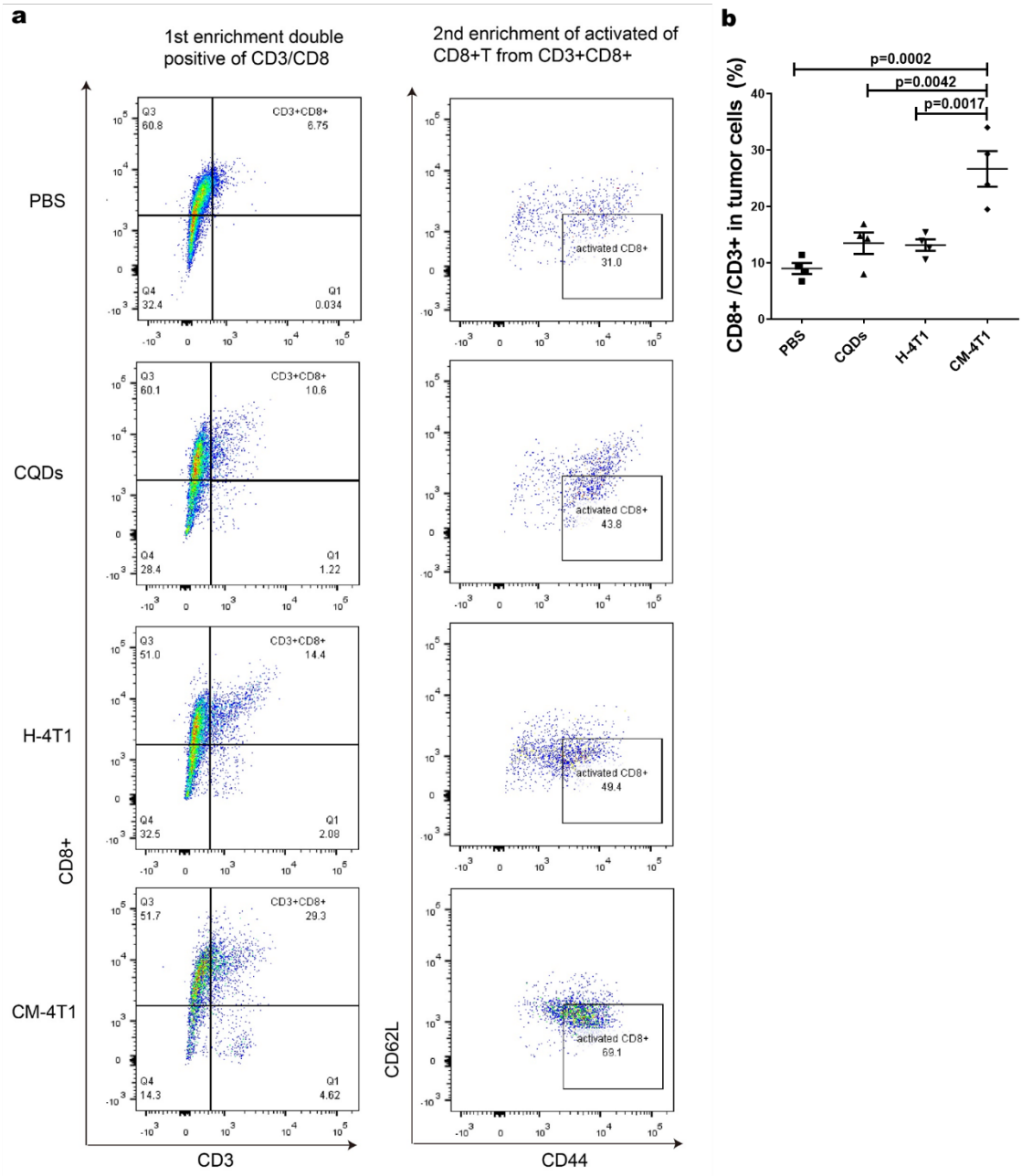
Supplementary Fig. 18. Images of 4T1 primary tumors in forth mammary glands in P0-P4 groups after PBS, H-4T1, CM–EMT6, CM–4T1 and CQDs treatments, respectively. The mice were sacrificed on day 29 and measured tumor size.



Supplementary Fig. 19. H&E staining of heart, liver, spleen and kidney from P0-P4 groups of 4T1 murine mammary model mice. Scale bar: 200 μm. The mice were sacrificed on day 29 and performed H&E staining.



Supplementary Fig. 20. H&E staining of liver tissues from M0-M4 groups after PBS, CQDs, H-4T1, CM-EMT6 and CM-4T1 treatments, respectively. Scale bar: 100 μm**.** The mice were sacrificed on day 29 and performed H&E staining.



Supplementary Fig. 21. Representative flow cytometry plots (a) and ratios (b) of activated CD8+ T cells in $CD8^+/CD3^+$ (%) in the tumors from P0-3 groups after PBS, CQDs, H-4T1 and CM-4T1 treatments (*n*=4), respectively; Data plotted are mean ± S.E.M. The mice were sacrificed on day 29 and performed flow cytometry.

Supplementary Table. 1 Antibodies used for experiments.

Item Description	Source	Model / Cat. #	IHC/IF
CD8α (D8A8Y) Rabbit mAb	CST	#85336	1:400
F4/80 Monoclonal Antibody (BM8)	eBioscience™	#14-4801-82	1:100
Cleaved Caspase-3 (Asp175) Antibody	CST	#9661	1:1000
Recombinant Anti-Ki67 antibody [SP6]	Abcam	ab16667	1:250
CD11c (D1V9Y) Rabbit mAb	CST	#97585	1:400
Anti-Interferon gamma antibody	Abcam	ab216642	1:200

Supplementary Table 2 Antibodies used for experiments.

Item Description	Source	Model / Cat. #	Flow
CD3e Monoclonal Antibody (145-2C11), Alexa Fluor 488	ThermoFisher Scientific	Cat# 53-0031-82; RRID:AB_469889	1:1000
CD8a Monoclonal Antibody (53-6.7), PerCP-eFluor 710	ThermoFisher Scientific	Cat# 46-0081-82; RRID:AB_1834433	1:1000
CD44 Monoclonal Antibody (IM7), PE	ThermoFisher Scientific	Cat# 12-0441-82; RRID:AB_465664	1:1000
CD62L (L-Selectin) Monoclonal Antibody (MEL14), eFluor 450	ThermoFisher Scientific	Cat# 48-0621-82; RRID:AB_1963590	1:1000
F4/80 Monoclonal Antibody (BM8), eFluor 450	ThermoFisher Scientific	Cat# 14-4801-82; RRID:AB_1548747	1:1000
CD206 (MMR) Monoclonal Antibody (MR6F3), APC	ThermoFisher Scientific	Cat# 17-2061-82; RRID AB_2637420	1:1000