

## Supporting Information

### Combinatorial Photothermal and Immuno Cancer Therapy Using Chitosan-Coated Hollow Copper Sulfide Nanoparticles

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#### Materials and Methods

**Synthesis of thiolated chitosan.** Thiolated chitosan was synthesized by modifying the previously reported method.<sup>1</sup> Chitosan (medium molecular weight) solution was prepared by dissolving 200 mg chitosan in 20 mL of 1% (v/v) acetic acid solution to which 160  $\mu$ L of thioglycolic acid was added. Then, 0.2 g of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride was added to the solution so as to activate the carboxylic acid moieties of TGA. The pH of the whole solution was adjusted to 5.0 using 5 mol/L of NaOH solution. The solution was stirred for 6 h at room temperature. The obtained thiolated chitosan was purified through ultra-filtration (molecular weight cut-off 30 kDa, Millipore) followed by lyophilization. The final product was stored at -20 °C.

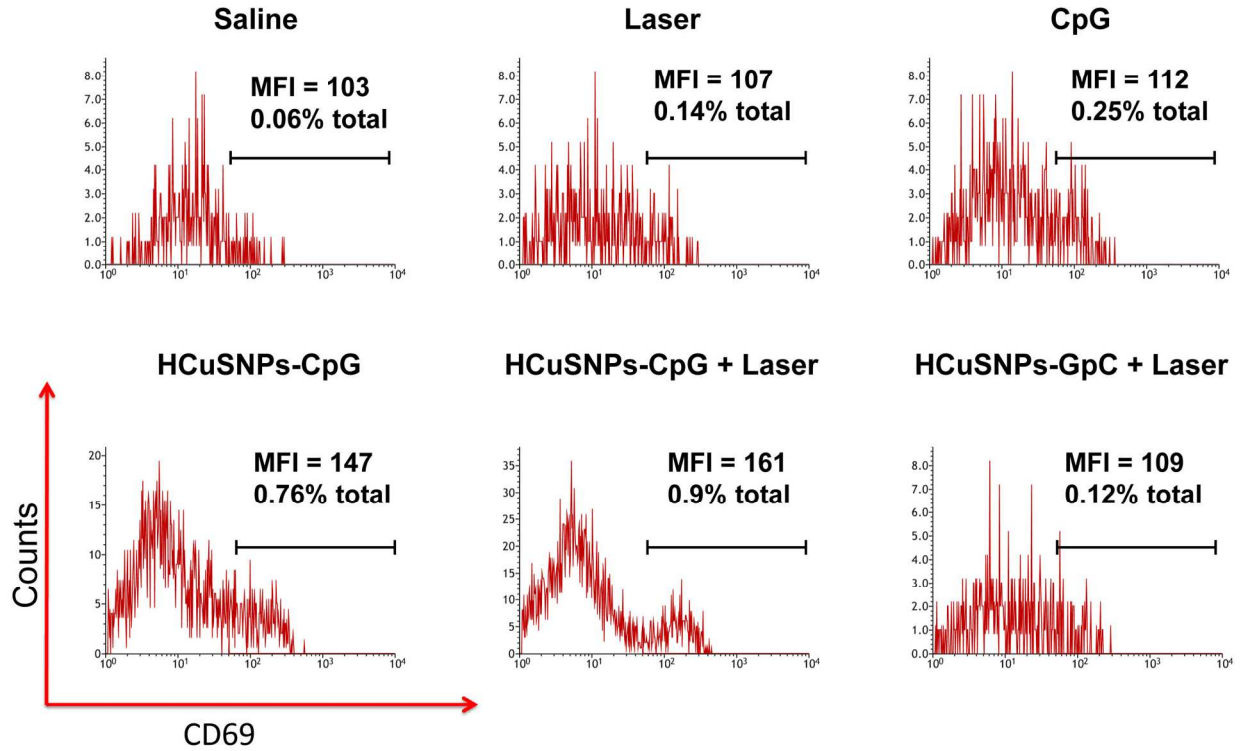
#### EMT6-OVA and characterization.

EMT6 cells were transfected with pCl-neo-OVA (Addgene) using Lipofectamine 2000 (Life Technologies) according to manufacturer's protocol. The positive clones, *i.e.* ovalbumin (OVA)-transfected EMT6 (EMT6-OVA) cells, were isolated by limiting-dilution analysis as previously described.<sup>2</sup> The EMT6-OVA cells were cultured in complete medium consisting of Waymouth, G 418

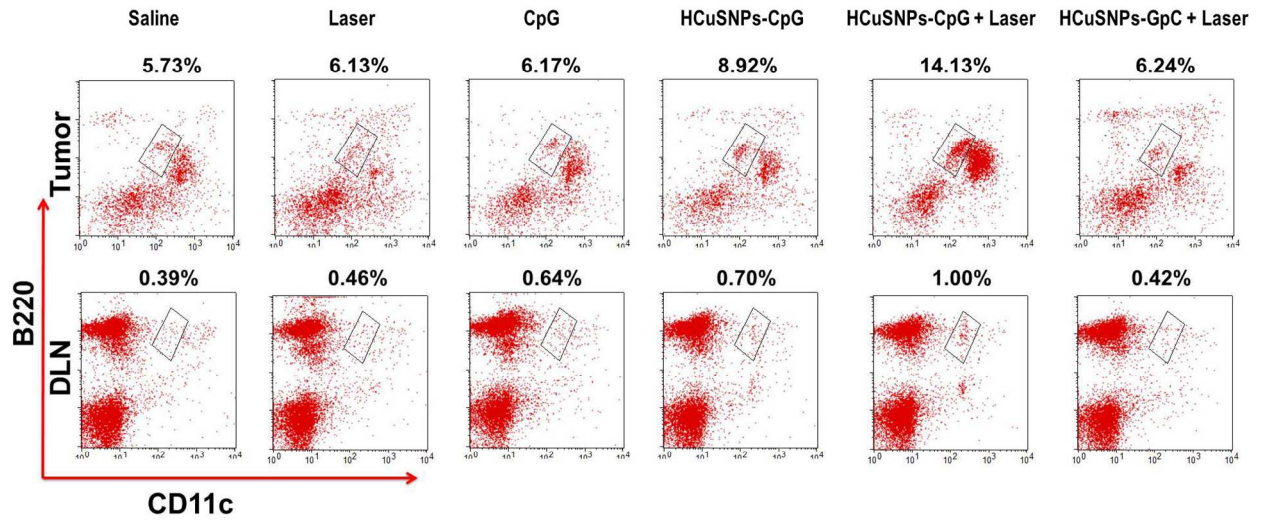
disulfate salt (200 µg/mL) (Sigma), penicillin (100 U/mL), streptomycin (100 µg/mL) and 10% FBS. The EMT6-OVA cells were characterized by Western blotting analysis of OVA expression. Primary antibodies: mouse anti-OVA polyclonal antibody (Thermo Scientific) at a dilution of 1:1000 and mouse anti-GAPDH monoclonal antibody (Sigma-Aldrich) at a dilution of 1:5000. Secondary antibody: IRDye680 goat anti-mouse IgG (Li-Cor) at a dilution of 1:10,000. After washing, the fluorescence in the membrane was visualized under an Odyssey imaging system (Li-Cor).

**Flow cytometric analysis of intracellular IFN- $\gamma$  production by CD8<sup>+</sup> T cells in tumor.**

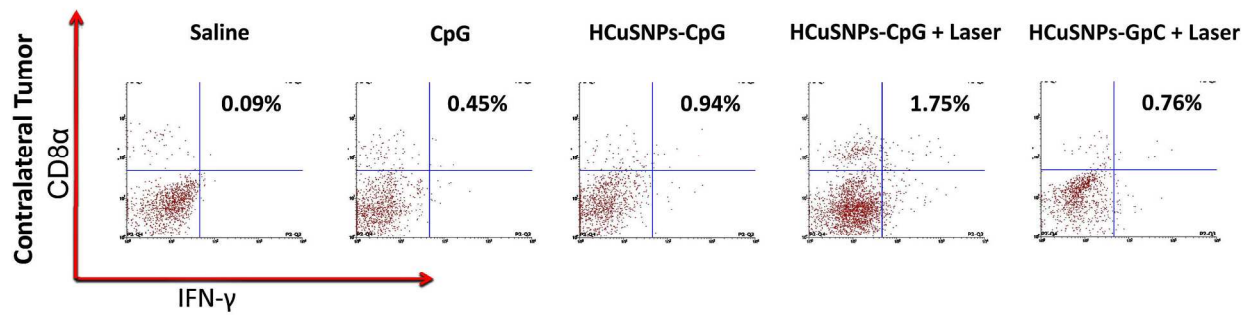
EMT6-OVA tumor-bearing mice were used to assess the antigen-specific T cell response. Briefly,  $1 \times 10^6$  EMT6-OVA cells were s.c. inoculated in right flank of the mice. When tumor size reached 4-6 mm, the mice were treated with intratumoral injection of saline, HCuSNPs-CpG (100 µg of CpG), or HCuSNPs-CpG (100 µg of CpG) plus laser irradiation at Day 0 and Day 6, respectively. At Day 12, the mice were euthanized to obtain the tumor cell suspension. After stimulation with 5 µg/mL of OVA in the presence of BD GolgiStop™ (Protein Transport Inhibitor, BD sciences) for 6 h *in vitro*, the cells were collected and incubated with anti-mouse CD16/32 to block unspecific binding and stained with anti-CD8 $\alpha$  and anti-IFN- $\gamma$  antibodies according to the manufacturer's instructions (eBioscience).



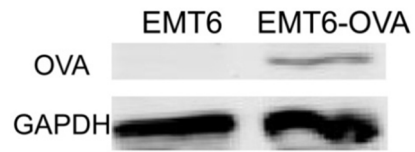
**Figure S1.** EMT6 tumor-bearing mice were given intratumoral injection of saline or different formulation containing 100  $\mu\text{g}$  of CpG or GpC (sham CpG control) with or without laser treatment ( $2.0 \text{ W/cm}^2$ , 40 s, 900 nm) at Day 0 and Day 6. Flow cytometric analysis of CD69<sup>+</sup> cells in lymphocyte population in tumor. MFI represents median fluorescence intensity of the gated cells. “% total” represents the percentage of the gated cells in total cells of tumor. “HCuSNPs-GpC” represents HCuSNPs-chitosan-GpC conjugates.



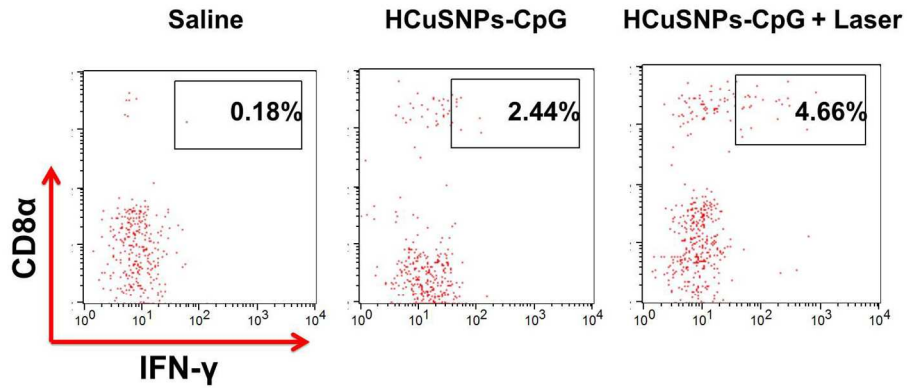
**Figure S2.** EMT6 tumor-bearing mice were given intratumoral injection of saline or different formulation containing 100  $\mu\text{g}$  of CpG or GpC (sham CpG control) with or without laser treatment ( $2.0 \text{ W/cm}^2$ , 40 s, 900 nm) at Day 0 and Day 6. Flow cytometric analysis of plasmacytoid dendritic cells ( $\text{CD11c}^{\text{int}}\text{B220}^+$ )<sup>3</sup> in tumor or draining lymph nodes (DLNs) at Day 8. “HCuSNPs-GpC” represents HCuSNPs-chitosan-GpC conjugates.



**Figure S3.** BALB/c mice bearing EMT6 tumor in the right flank were given intratumoral injection of saline or different formulation containing 100  $\mu\text{g}$  of CpG or GpC (sham CpG control) with or without laser treatment ( $2.0 \text{ W/cm}^2$ , 40 s, 900 nm) at Day 0 and Day 6. At Day 6, the mice were s.c. injected with  $3 \times 10^5$  EMT6 cells in the left (contralateral) flank for tumor challenge. Flow cytometric analysis of intracellular IFN- $\gamma$  production by CD8<sup>+</sup> T cells in the contralateral tumor at Day 16 followed by CpG (20  $\mu\text{g/mL}$ ) stimulation *in vitro*. “HCuSNPs-GpC” represents HCuSNPs-chitosan-GpC conjugates.



**Figure S4.** Western blot analysis of OVA expression in EMT6 cells and EMT6 cells stably transfected with pCl-neo-OVA (EMT6-OVA).



**Figure S5.** EMT6-OVA tumor bearing mice were given intratumoral injection of saline or HCuSNPs-CpG (100  $\mu$ g of CpG) with or without laser treatment (2.0 W/cm<sup>2</sup>, 40 s, 900 nm) at Day 0 and Day 6. Flow cytometric analysis of intracellular IFN- $\gamma$  production by CD8<sup>+</sup> T cells in tumor following OVA (5  $\mu$ g/mL) stimulation *in vitro* at Day 12.

## References

1. Lee, D.; Zhang, W.; Shirley, S. A.; Kong, X.; Hellermann, G. R.; Lockey, R. F.; Mohapatra, S. S. Thiolated Chitosan/DNA Nanocomplexes Exhibit Enhanced and Sustained Gene Delivery. *Pharm Res* **2007**, *24*, 157-167.
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3. Liu, C.; Lou, Y.; Lizee, G.; Qin, H.; Liu, S.; Rabinovich, B.; Kim, G. J.; Wang, Y. H.; Ye, Y.; Sikora, A. G., *et al.* Plasmacytoid Dendritic Cells Induce NK Cell-Dependent, Tumor Antigen-Specific T Cell Cross-Priming and Tumor Regression in Mice. *J Clin Invest* **2008**, *118*, 1165-1175.