

## SUPPORTING INFORMATION

# Tumor-Selective Immune Active Mild Hyperthermia Associated with Chemotherapy in Colon Peritoneal Metastasis by Photoactivation of Fluorouracil – Gold Nanoparticle Complexes

*Vladimir Mulens-Arias,<sup>1,3†</sup>, Alba Nicolás-Boluda,<sup>1†</sup> Amandine Pinto,<sup>2</sup> Alice Balfourier,<sup>1</sup>*

*Florent Carn,<sup>1</sup> Amanda K. A. Silva,<sup>1</sup> Marc Pocard,<sup>2</sup> and Florence Gazeau<sup>1\*</sup>*

<sup>1</sup> Université de Paris, Laboratoire MSC Matière et Systèmes Complexes, CNRS UMR 7057,  
10 Rue Alice Domon et Léonie Duquet, 75205 Paris Cedex 13, France

<sup>2</sup> Université de Paris, UMR 1275 CAP Paris-Tech, F-75010 Paris, France; Service de chirurgie  
digestive et cancérologique Hôpital Lariboisière 2 rue Ambroise Paré, F-75010 Paris, France.

<sup>3</sup>Department of Immunology and Oncology, National Center for Biotechnology/CSIC, Darwin  
3, Cantoblanco Campus, 28049 Madrid, Spain

## Supplementary Materials & Methods

The RAW264.7 (ATCC® TIB-71™), HuH7 (ThermoFisher), A549 (ATCC® CCL-185™), and MDA-MB-231 (ATCC® HTB. 26™) cells were cultured in complete DMEM supplemented with 10% fetal bovine serum (FBS), L-glutamine, and penicillin/streptomycin. Subconfluent cells were incubated for 24 h with AuNP or freshly prepared AuNP-5FU (as described in M&M section) in complete DMEM. For the last 4 h, the Presto Blue cell viability reagent was added (1/10 final dilution; Presto Blue cell viability reagent, A13261; Invitrogen). Fluorescence was measured with a 535 nm excitation filter and a 615 nm emission filter. The fluorescence of complete DMEM incubated with Presto Blue was used as the background control. Cell viability was calculated according to the formula:

$$Viability (\%) = \frac{[(Fluorescence_{Autreatedcells}) - (Fluorescence_{medium})]}{[(Fluorescence_{untreatedcells}) - (Fluorescence_{medium})]} * 100\%$$

### List of Supporting Figures:

**Figure S1.** TEM image and size distribution of 16nm gold nanoparticles. A) TEM images of AuNPs. B) Gold core diameters as measured by image analysis of TEM.

**Figure S2.** Structural characterizations of the complexation process between 16 nm AuNP and 5-FU during the first 24h after mixing. (A), Cryo-TEM imaging of as-formed nanostructures within the first 24 h. Scale: 50 nm (B), Nanoparticle numbers per complex and complex diameter as measured from cryo-TEM images.

**Figure S3.** 5-FU loading onto AuNP nanoclusters as determined in Materials and Methods. The left bar shows the averaged 5-FU amount per 40 µg of Au. The right bar represents the corresponding % loading. Bars represent the average of 29 conjugation reactions.

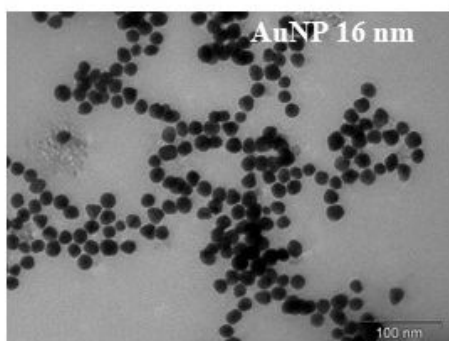
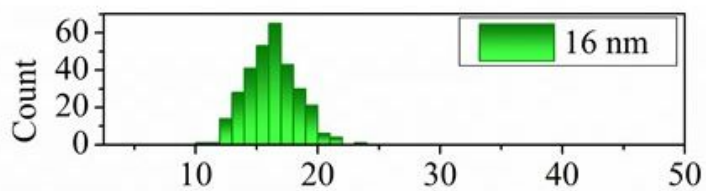
**Figure S4.** Cytotoxicity of AuNP, AuNP-5FU complex, and equivalent 5-FU against different cell types as determined in Materials and Methods. (A) Schematic of experiment procedure. (B) Viability of cells upon 24h treatment.

**Figure S5.** Biodistribution of Au in spleen, kidneys, liver, lungs, heart, and tumors after intratumoral injection of 40 µg of Au intratumorally. Percentage of injected dose per organ (n=5 per group). Results are represented as mean ± SEM

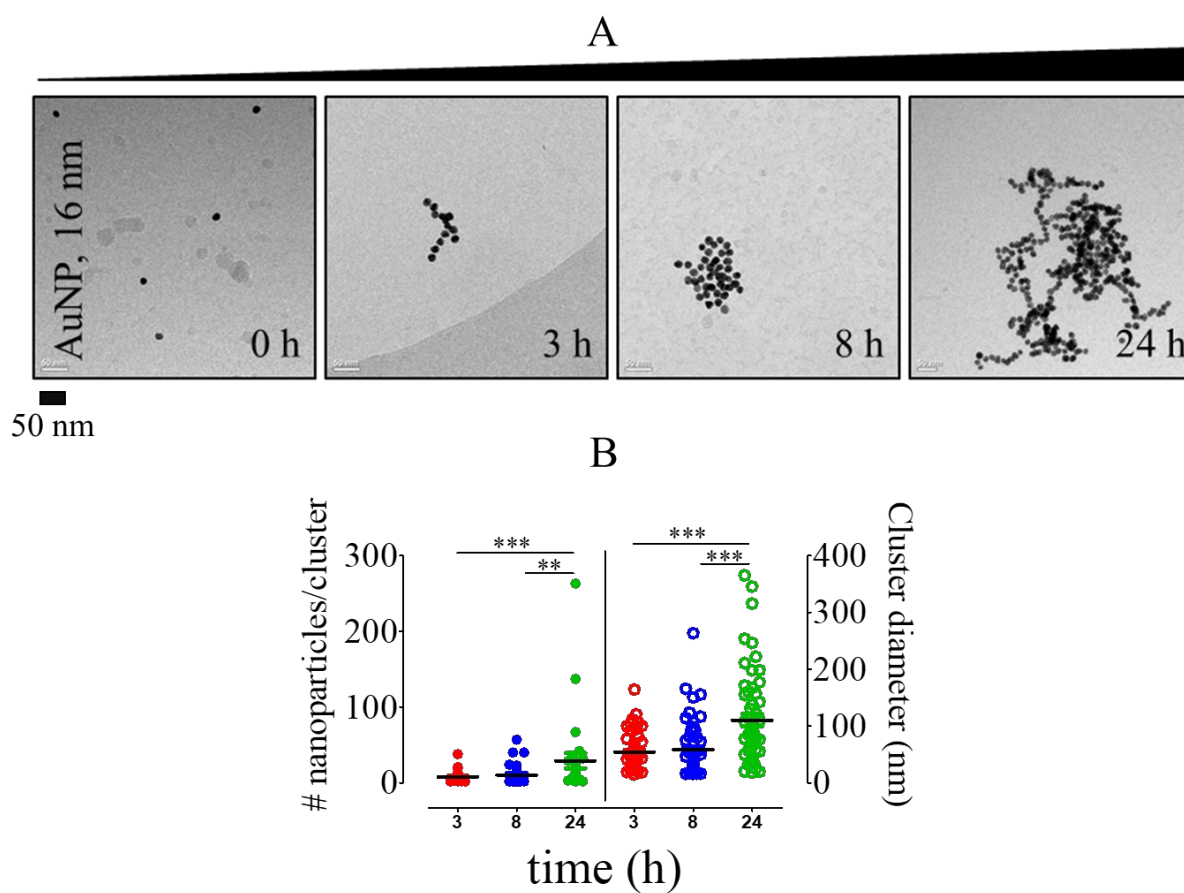
**Figure S6.** Gating strategy to analyze the phenotype of peritoneal macrophages.

**Figure S7.** A) Percentage of alive peritoneal cells in each of the conditions. B) Percentage of CD45+ cells in the peritoneal cavity in each of the conditions.

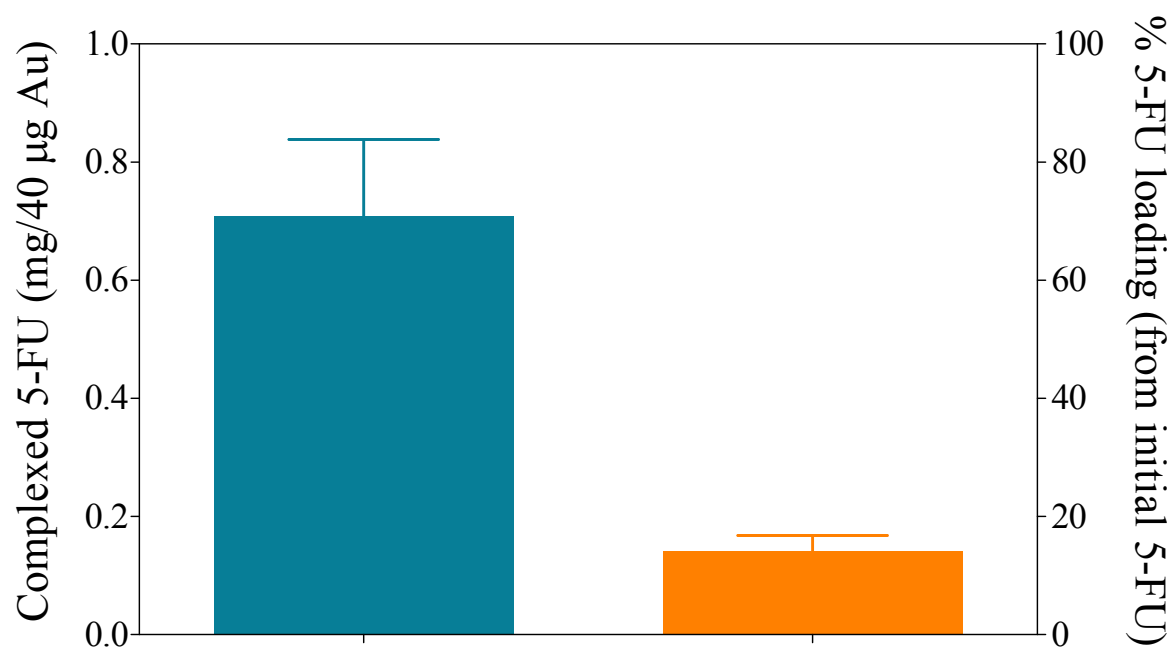
**Figure S8.** Biodistribution of Au in kidneys, liver, spleen, intestine, and tumor after injection of AuNP and AuNP-5FU intraperitoneally. Injected dose of AuNP and AuNP-5FU was [Au] = 120 µg. Percentage of the injected dose per organ (n = 5 per group). Results are represented as mean ± SEM.

**A****B**

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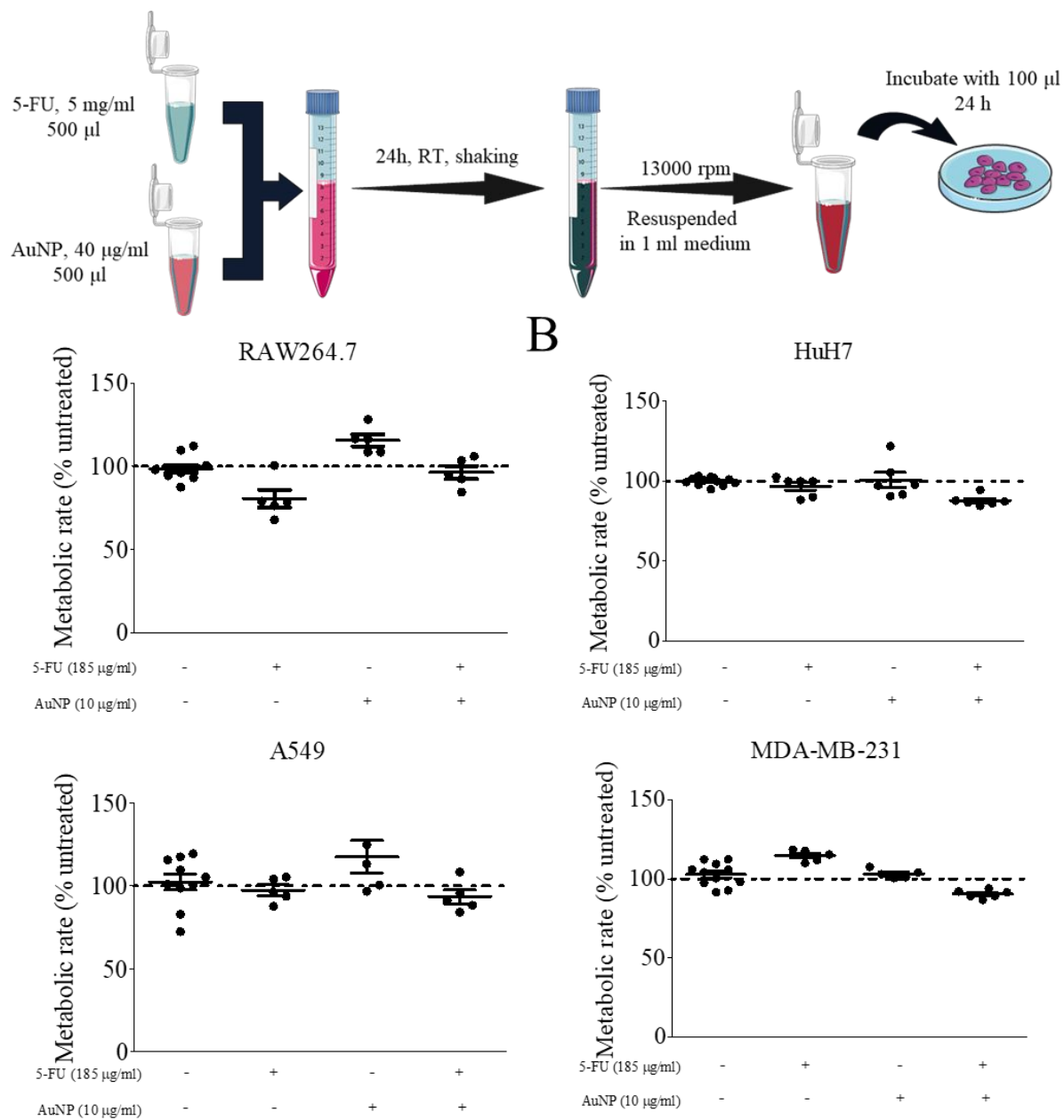


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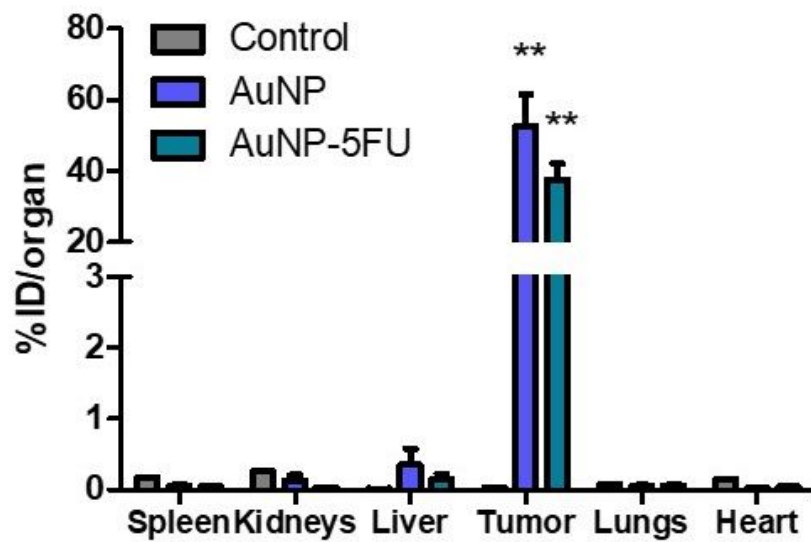


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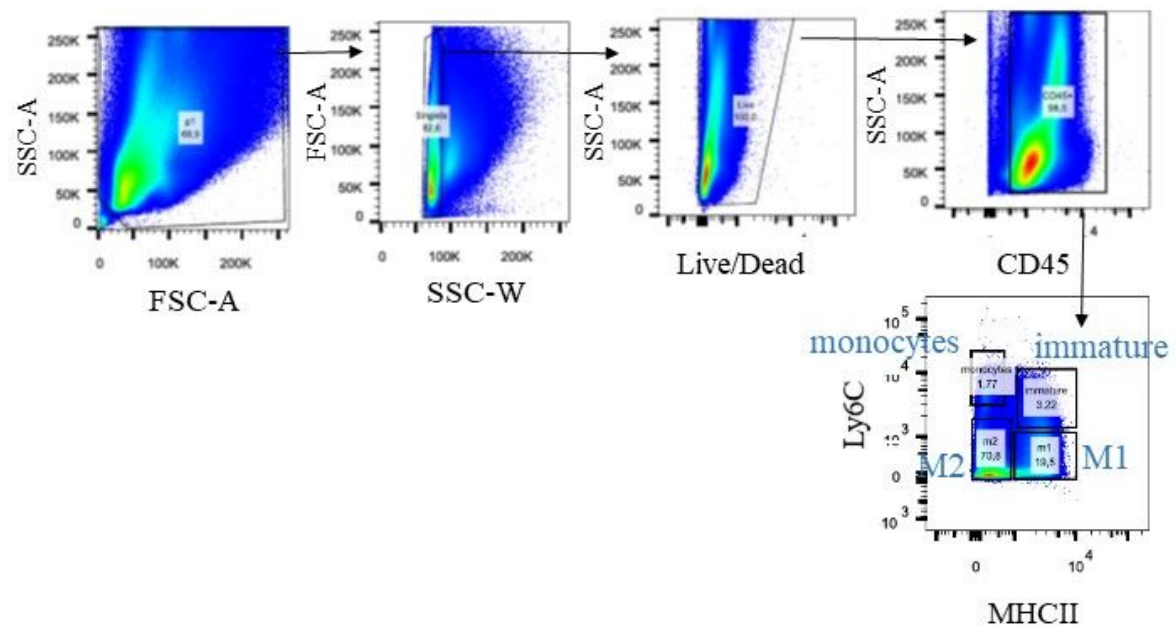


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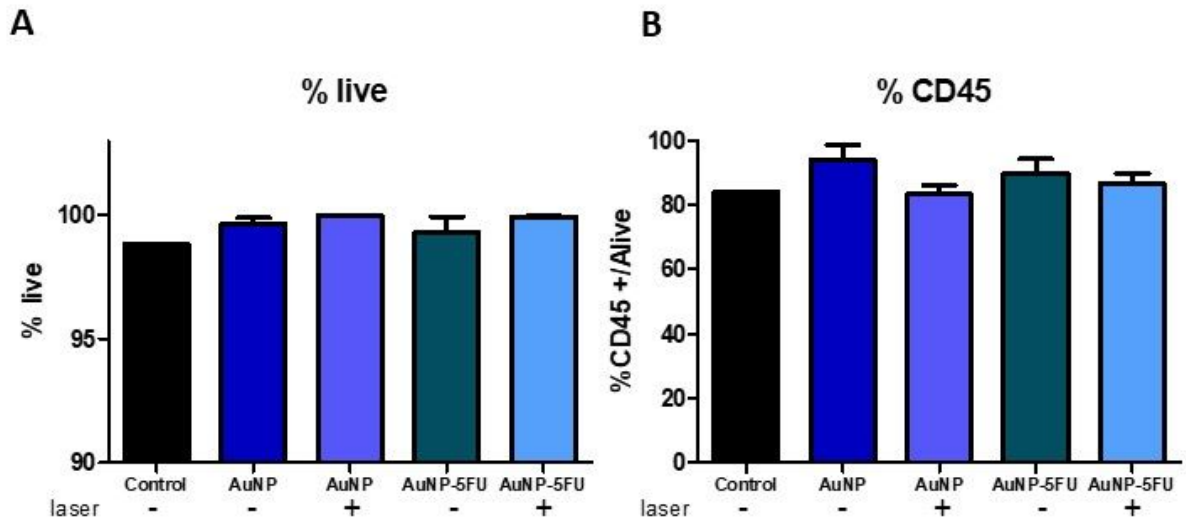


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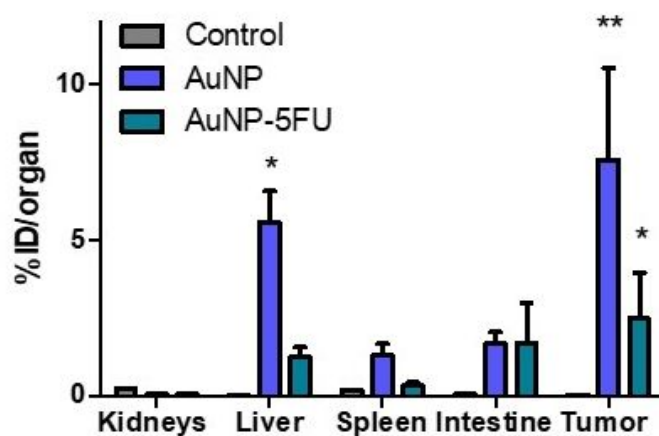




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