Supporting Information

Hexahistidine-metal Assemblies: A Facile and Effective Co-delivery System of Subunit Vaccines for Potent Humoral and Cellular Immune Responses

Tinghong Zhang, [a, b] *Long Zhang,* [b] *Xiaoxiao Wu,* [a] *Hongyan Xu,* [a] *Pengyan Hao,* [a] *Wenjuan Huang,* [a] *Yagang Zhang* [c] *Xingjie Zan* [a, b]*

[a] School of Ophthalmology and Optometry, Eye Hospital, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou 325035, P. R. China

[b] Wenzhou Institute, University of Chinese Academy of Sciences (Wenzhou Institute of Biomaterials & Engineering), Wenzhou 325001. P. R. China.

[c] School of Materials and Energy, University of Electronic Science and Technology of China, Chengdu 611731, China

Corresponding author: Xingjie Zan, E-mail: xjzan2000@hotmail.com.

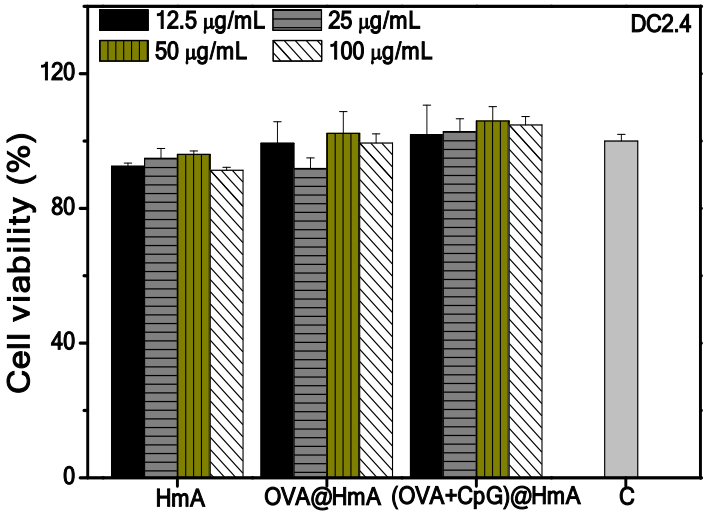

Figure S1. Viability of DC2.4 cells, measured by CCK-8 assay after treatment with increasing concentrations of HmA, OVA@HmA, and (OVA+CpG)@HmA for 24 h.

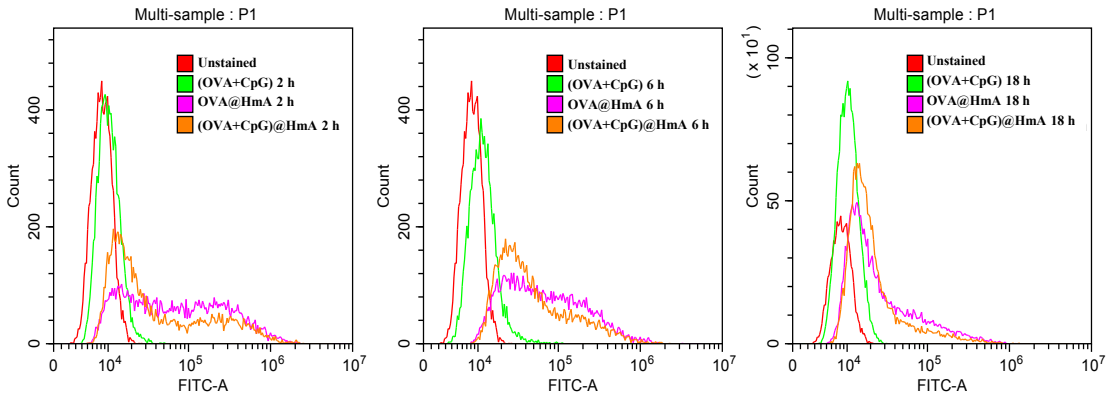

Figure S2. Representative images of cellular uptake by flow cytometry. RAW264.7 cells were treated with different samples for indicated times (2, 6 and 18 h). Cellular uptake was assessed by flow cytometry assay.

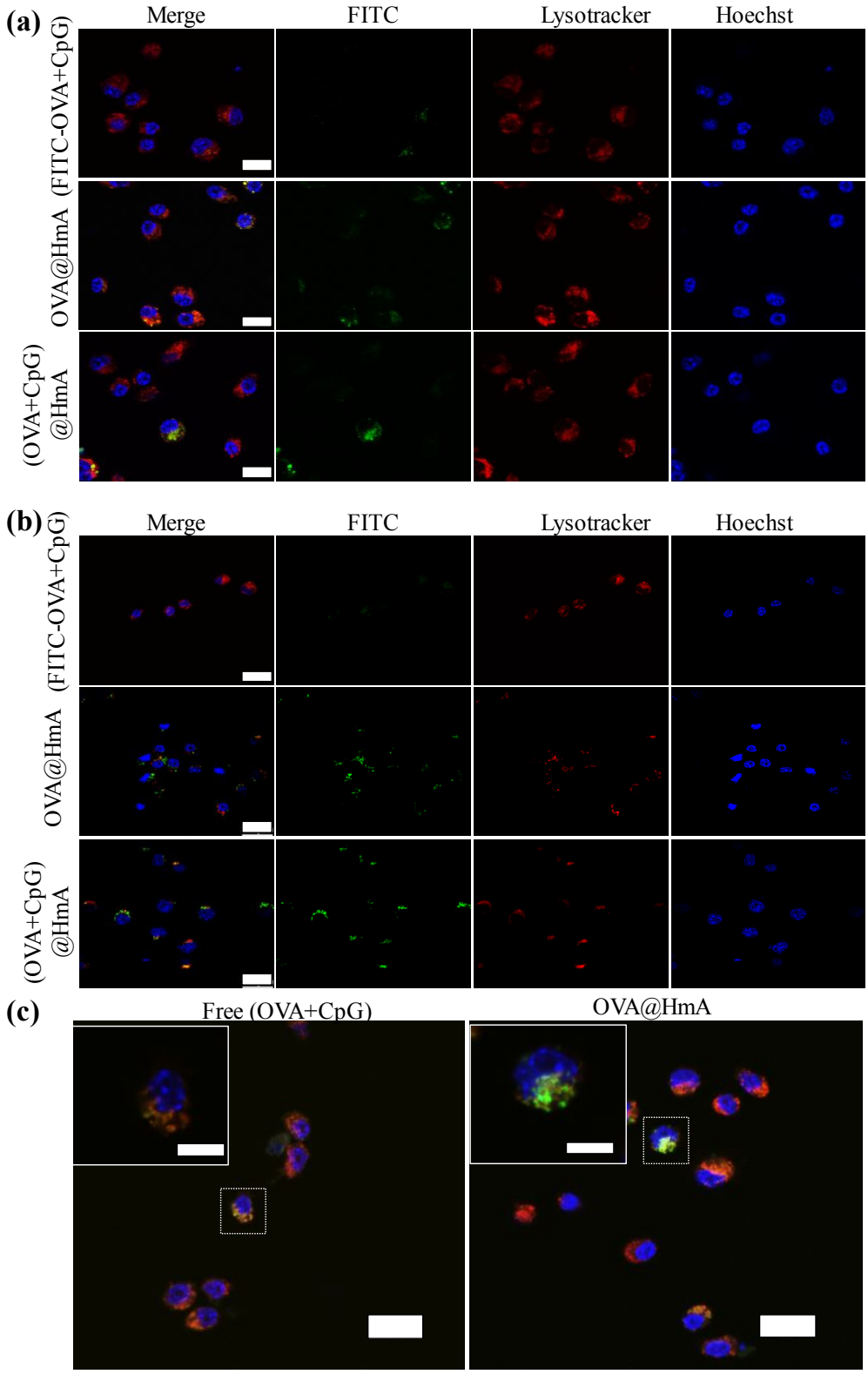

Figure S3. Cellular uptake of various samples in RAW264.7 cells by CLSM. CLSM images of RAW 264.7 cells after incubation with different simples for 2 h (a) and 18 h (b). (c) Enlarged CLSM image of RAW 264.7 cells after incubation with different

simples for 6 h. Lysosome was stained with Alexa Fluor 555 conjugated Lysotracker (red), and the cell nucleus with Hoechst (blue), OVA peptides were labeled with FITC (green). Scale bar: 20 μm.

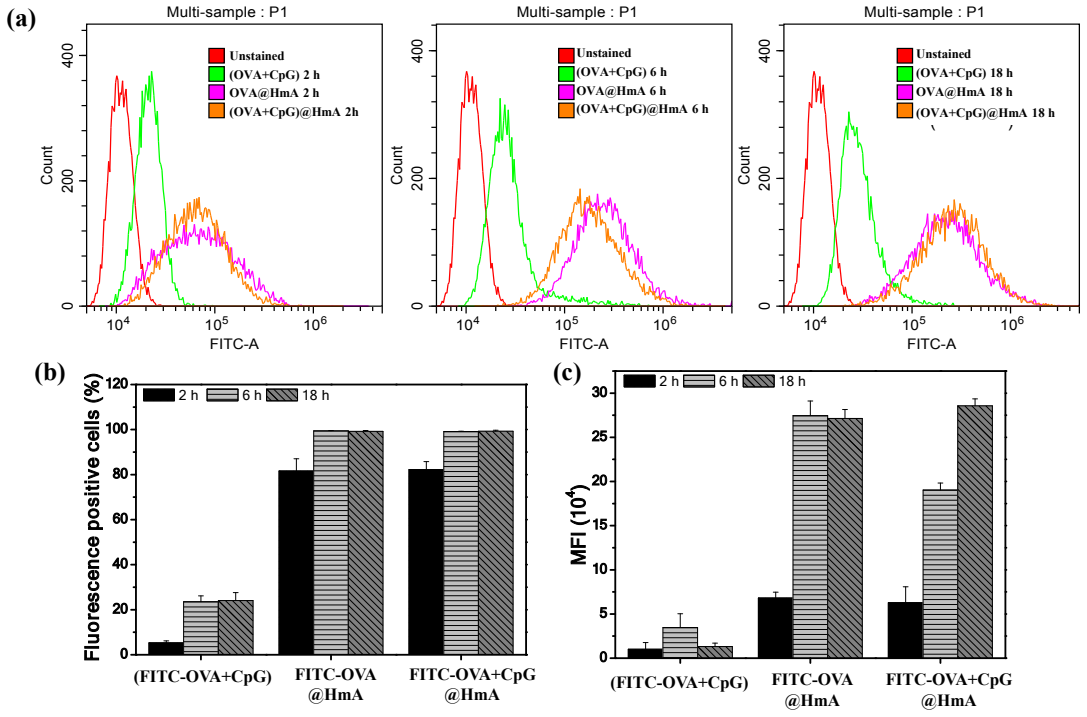

Figure S4. Cellular uptake of various samples in DC2.4 cells. (a) Representative scatter diagram of flow cytometry. (b) Fluorescence positive cells and (c) mean fluorescence intensity of DC 2.4 cells after incubated with different simples for 2, 6 and 18 h quantified by flow cytometry.

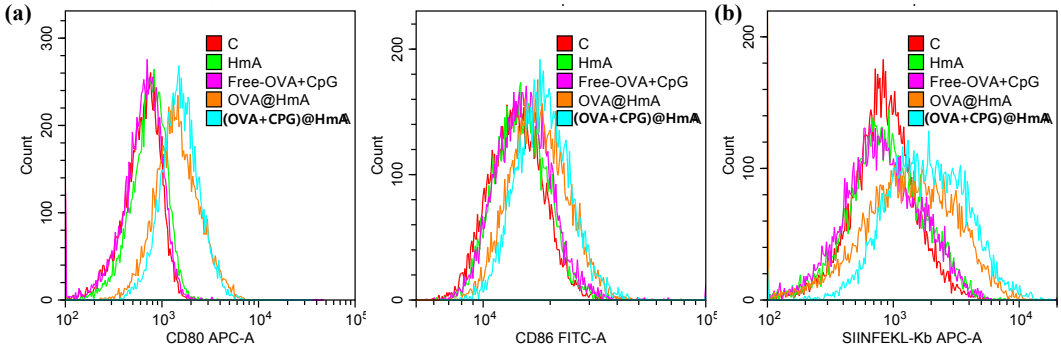

Figure S5. The expression of costimulatory molecules (CD80 and CD86) (a) and the cross-presentation of antigen (b) on RAW264.7 cells detected by flow cytometry.

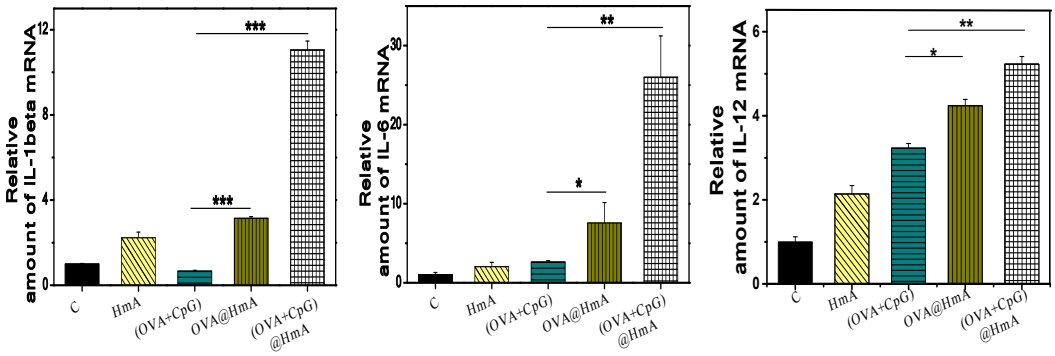

Figure S6. The mRNA levels of cytokines (IL-1β, IL-6 and IL-12) in RAW264.7 after incubated with different samples for 24 h. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.