Supporting information

In Vivo Imaging Tracking and Immune Responses to Nanovaccines Involving Combined Antigen Nanoparticles with a Programmed Delivery

Xia Dong[1#], Jie Liang[1#], Afeng Yang[1], Chun Wang[1,2], Deling Kong[1], Feng Lv[1]*

1 Tianjin Key Laboratory of Biomedical Materials, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, 300192, PR China

2 Department of Biomedical Engineering, University of Minnesota, Minnesota 55455, United States

# These authors contributed equally to this work and should be considered co-first authors.

*Corresponding author:

Feng Lv

Institute of Biomedical Engineering

Chinese Academy of Medical Sciences & Peking Union Medical College

Tianjin 300192, PR China

Tel/Fax: 86-22-87893236, E-mail: lvfeng2002@163.com (Lv F)

Materials and methods

1. Materials

Ovalbumin (OVA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Roswell ParkMemorial Institute (RPMI) 1640 and fetal bovine serum (FBS) were supplied by Gibco (Grand Island, NY, USA). Lyso-Tracker Red probes and DAPI Staining Solution were approved by Beyotime Biotechnology (Beyotime, Shanghai, China). Mouse cytokine ELISA kits and fluorochrome-labeled MHC I, CD40, CD86, CD80, CD11c, CD44, CD62L, CD4, and CD8a antibodies were obtained from eBioscience (San Diego, CA, USA). Recombinant mouse GM-CSF and IL-4 were obtained from Peprotech (Rocky Hill, NJ, USA). PE-labeled antimouse intracellular interferon-γ (IFN-γ) antibodies were obtained from BD Biosciences (San Jose, CA, USA). Genipin (98%) was purchased from Xi'an Plant Bio-engineering Corporation (Xi'an, China). G418 was obtained from Beijing Solarbio Science & Technology (Beijing, China). PEI_{10000} was approved by Aladdin Chemistry Corporation (Shanghai, China). All other reagents were of analytical grade.

2. Cell culture and animals.

Dendritic Cells (DCs) were cultured from bone marrow cells of C57BL/6 mice. The bone marrow cells (BMDCs) were obtained by flushing the femur and tibia and cultured in RPMI medium1640 supplemented with GM-CSF (20 ng/mL) and IL-4 (10 ng/mL) at 37 °C. After 6 days, DCs were obtained, washed, and used for in vitro experiments. E.G7-OVA cells were cultured in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, 10 mM HEPES and 1.0 mM sodium pyruvate and supplemented with 0.05 mM 2-mercaptoethanol and 0.4 mg/ml G418.

Male 6-week-old C57BL/6 mice were housed with free access to water and food. All the animal procedures were carried out according to the protocol approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Laboratory Animal Ethics Committee, Peking Union Medical College, People's Republic of China. All the animal experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Peking Union Medical College, China.

3. Preparation and characterization of OVA NPs and combined OVA NP

OVA Nanoparticles (OVA NPs) was prepared by genipin cross-linked reaction between OVA and PEI. Genipin solution (1% w/v) was added to the OVA sulution(5mg/mL) to stir over night and the color of the system changed to slight yellow after 12h. Then 1 mL of PEI solution (Mw = 10000 Da, 15mg/mL) was added to the system to stir for 48 h. After stirring, the solution was dialyzed (MWCO 3500Da) against de-ionized water for 24h to remove excess genipin. Then the product lyophilized to give the OVA NPs. The

combined OVA NPs (combined OVA NPs) was further prepared by electrostatic interaction with ova antigen on the surface of the OVA nanoparticles for 24h. In addition, the rhodamin B labeled OVA (OVA-RB) on OVA NPs was the same as OVA. The mass ratio of OVA or OVA-RB to OVA NPs was 1:1.

The free amino group content for the crosslinking degree of OVA NPs was analyzed by TNBS method. OVA NPs and the OVA/PEI mixed solution were dissolved in 1.0 ml of 0.1 M sodium phosphate buffer (pH 8.0), and 1ml of 2,4,6-Trinitrobenzenesulfonic acid (TNBS) solution (0.1% v/v) was added subsequently. Tubes were stored at 40 ◦C for 100 min and placed in the dark. The action was terminated by 50 μL of formic acid solution (88% v/v). Absorbance value was read at 424 nm by a multimode microplate spectrum photometer (VarioskanTM Flash, ThermoFisher Scientific, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used by electrophoresis apparatus trophoresis (DYCZ-24DN, Beijing Liuyi Biotechnology Corporation, China) to identificate free OVA and OVA NPs and proved the formation of OVA nanoparticles. Spatial structure of OVA NPs and combined OVA NPs were characterized by Circular Dichroism Chiroptical Spectrometer (J-815 CD spectrometer，JASCO，JAPAN). The morphology of the prepared nanoparticle was observed by atomic force microscopy (AFM) (Multi Mode8, Veeco Instruments, USA). The mean size, size distribution and zeta potential of the OVA NPs and combined OVA NPs were determined with a NY 90-Plus particle size analyzer and zeta potential analyzer (Nano ZS, Malvern Instruments, UK). UV−vis and fluorescence spectra of OVA NPs and combined OVA NPs were measured by a multimode microplate spectrum photometer (Varioskan^{TM} Flash, ThermoFisher Scientific, USA).

4. ELISA analysis of cytokine and levels of OVA-specific IgG and isotypes

OVA-specific serum antibodies (total IgG and IgG1, IgG2a subtypes) were measured by ELISA. Briefly, enzyme-linked ELISA assay plates (96 well) were coated with OVA (2 μg per well) in phosphate buffer overnight at 4 °C. The plates were blocked with 1% BSA after washed and incubated at 37℃ for 2 h. Then samples were serially diluted in blocking buffer, added to the plates, and incubated for 1 h at 37 °C. After that, the plates were incubated with HRP-conjugated goat anti-mouse IgG (IgG total, IgG1 or IgG2a) antibodies for 45 min at 37℃. HRP was quantified by treating TMB solution for 25 min at room temperature and the enzymatic reaction was stopped with stopping liquid. The absorbance at 450nm was measured using an ELISA reader and titers were given as the reciprocal sample dilution corresponding to twice higher OD than that of the negative sera. Cytokine TNF-α and IFN-γ were measured by ELISA with above similar method and the concentration of cytokine was determined from the absorbance at 450 nm. Samples were measured in triplicate.

Results

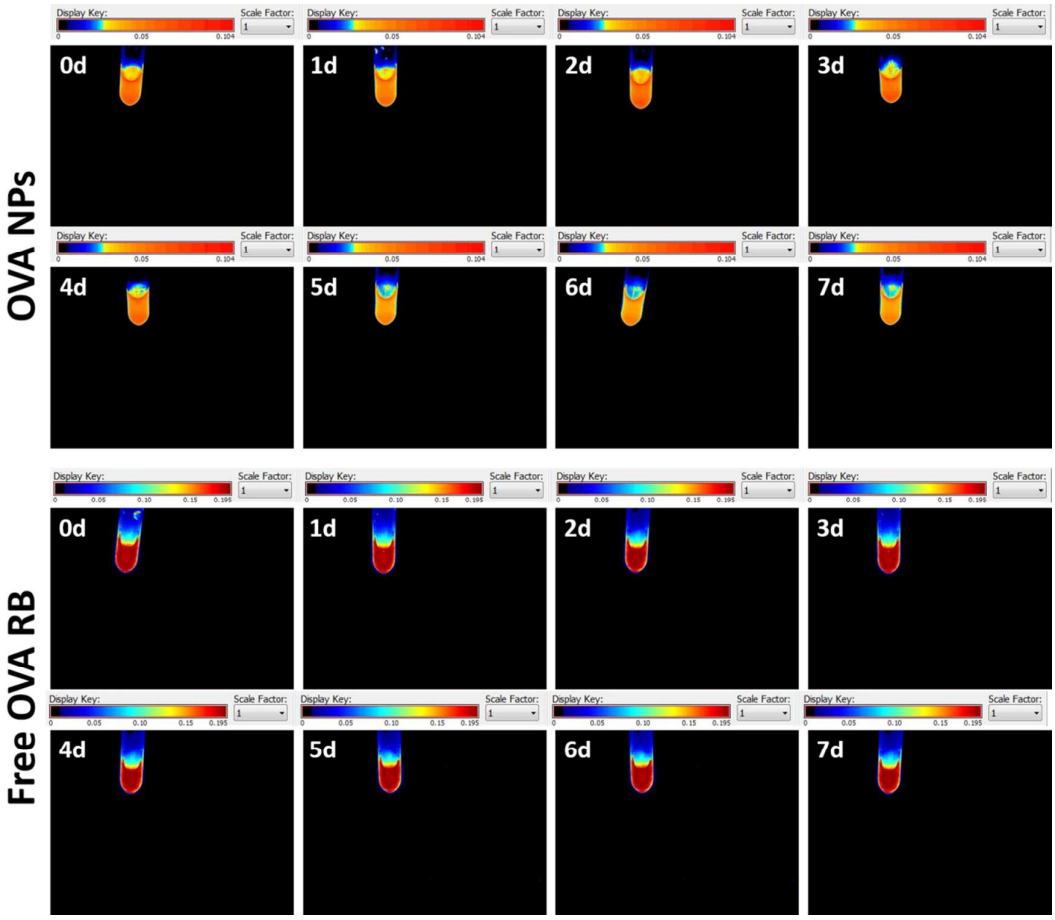

Figure S1Fluorescence imaging of OVA NPs and OVA-RB in vitro

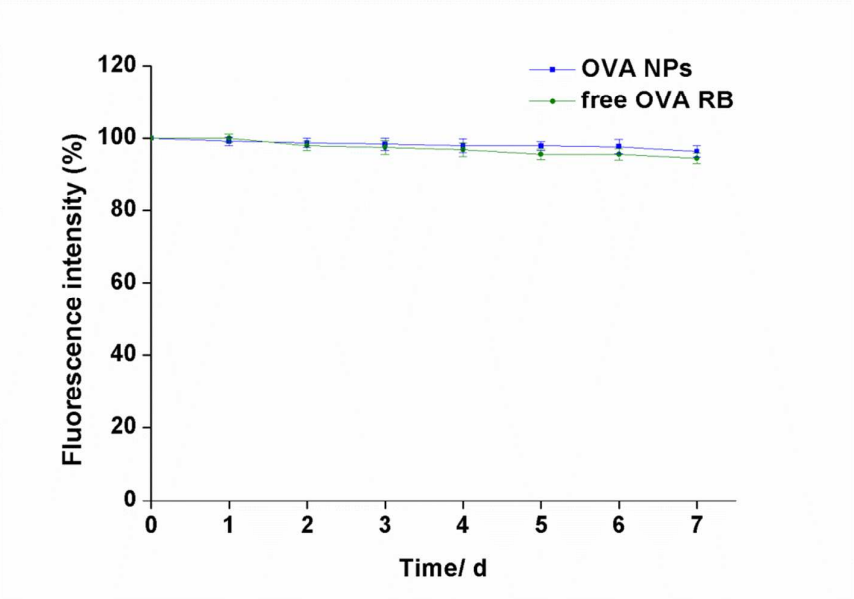

Figure S2 Quantitative analysis of fluorescence intensity of OVA NPs and OVA-RB in vitro

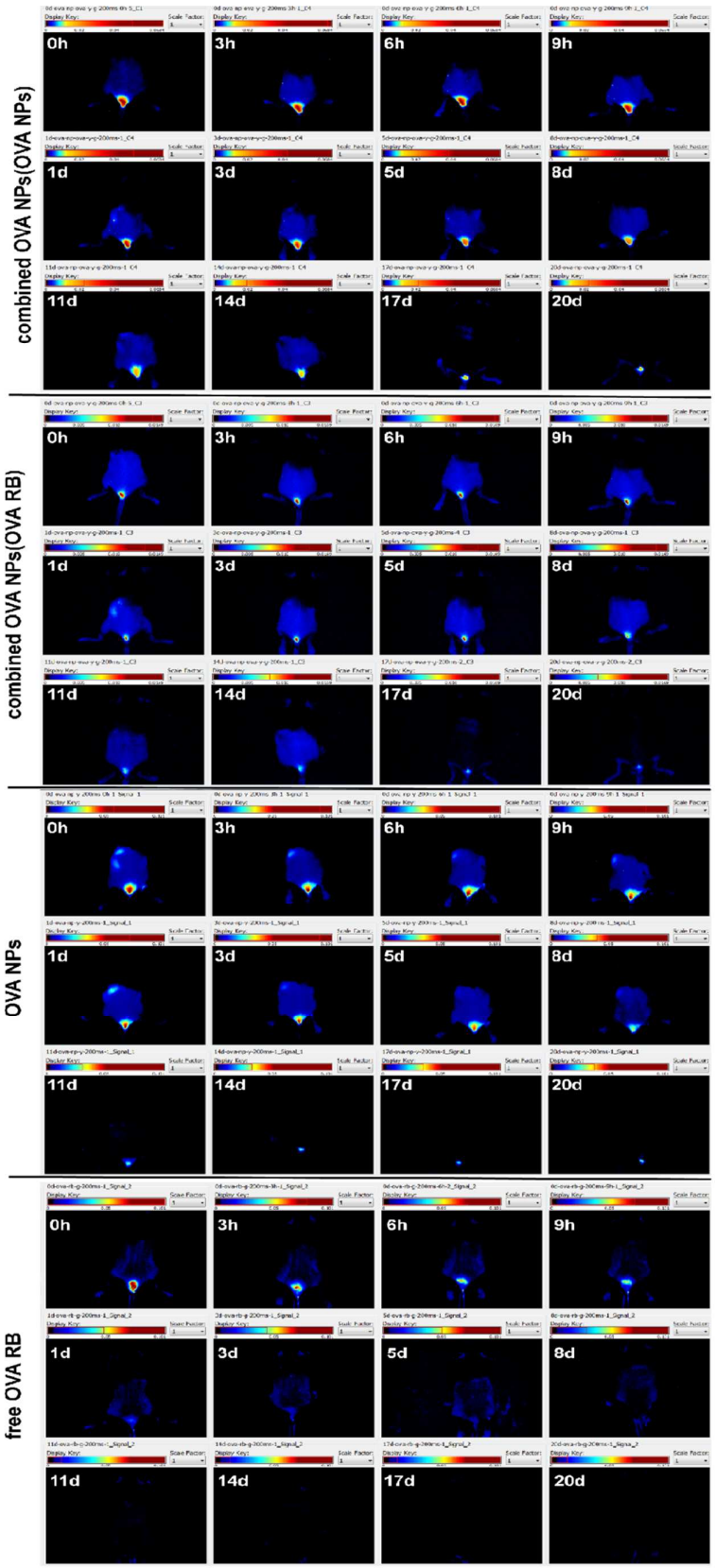

Figure S3 Fluorescence imaging tracking of free OVA RB, OVA-NPs or combined OVA-NPs with rainbow

color and one representative of three in each group,

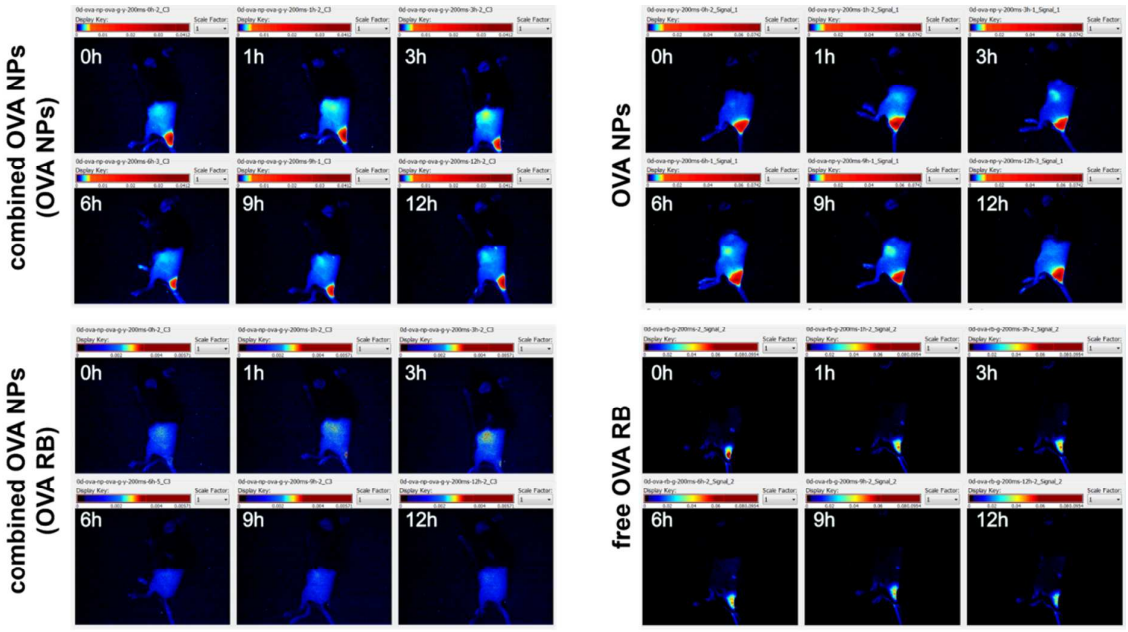

Figure S4 Antigen transport into draining lymph nodes from 0h to 12 h with compare function of Maestro software.