

Supporting Information

Antigen-enabled Facile Preparation of MOF Nanovaccine to Active Complement System for Enhanced Antigen-mediated Immune Response

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Experimental Section

Materials

Purified anti-mouse TNF- α , anti-mouse IFN- γ , anti-mouse IL-12 were purchased from Boster, China. Thiazolyl blue tetrazolium bromide (MTT) and ovalbumin (OVA) were purchased from Sigma-Aldrich (USA). Dulbecco's modified Eagle's medium (DMEM) and fetal Bovine Serum (FBS) were purchased from Invitrogen. Fibroblasts cell line (L929), hepatoma cell line (HpeG2), HeLa cell line and RAW 264.7 macrophages were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai).

Characterization

Transmission electron microscopy (TEM) images were recorded using a JEOL JEM-1011 electron microscope. UV-vis spectra were recorded on a Varian Cary 300 UV-visible spectrophotometer using cuvettes with 1 cm path-length. Fluorescent images of live and dead stained HeLa cells were taken by microscope (Cytation™ 5 Cell Imaging Multi-Mode Reader). Size and zeta potential of the nanoparticles were determined by dynamic light scattering (DLS) using Malvern Zeta-Sizer Nano. Confocal laser scanning microscopy (CLSM) images were taken using a Zeiss LSM 700 (Zurich, Switzerland). FTIR was measured by using a Nicolet Impact 410 Fourier transform infrared spectrometer. UV and fluorescence were recorded with a SHIMADZU UV-2450 and Edinburgh Instrument FLS-920 spectrometer, respectively. Powder X-ray diffraction (PXRD) was performed by using a Bruker D8 diffractometer using CuK α radiation, 40 kV, 40 mA with a scanning rate of 10 °C min⁻¹. Flow cytometry was carried out using a FlowSight® Imaging Flow Cytometer (Merck Millipore).

Synthesis of UiO-AM and UiO-OVA

UiO-AM with 50 mol% content of amine groups was synthesized by following our previous works.^{S1} To prepare UiO-OVA, UiO-AM was dissolved in Ultrapure water (1 mg mL⁻¹) followed by sonication for 1 h to form homo-disperse. The UiO-AM solution was then gently

mixed with different volume of OVA solution (1 mg mL^{-1}) in ultrapure water at $4 \text{ }^{\circ}\text{C}$ for 15 min to allow the encapsulation of OVA by the UiO-AM. The particle size and ζ potential of the UiO-OVA were determined. To determine the OVA loading capacity (LC) of the UiO, nanoparticles were ultra-centrifuged at $100,000 \text{ g}$ for 30 min, and the amount of bound OVA in UiO-OVA was determined using microwave-assisted heating. The LC calculated using the following equations:

$$\text{LC} = \text{UiO Residue Weight} - \text{UiO-OVA Residue Weight}$$

Preparation of OVA-FITC

OVA (10 mg) dissolved in sodium carbonate buffer ($\text{pH } 9.8$, $10 \text{ mL} \times 25 \text{ mM}$) was mixed with FITC ($200 \text{ } \mu\text{L} \times 1 \text{ mg mL}^{-1}$), and then mixed with FITC ($200 \text{ } \mu\text{L}$, 1 mg mL^{-1}) at $4 \text{ }^{\circ}\text{C}$ overnight under stirring. The product was spin-dialyzed through 30 kDa cut-off filter (Millipore), and dialyzed for 3 d. The final solution was stored at $-20 \text{ }^{\circ}\text{C}$ for future use.

Cytotoxicity Analysis

The cytotoxicity was determined by the viability of cells with MTT assay. L929, HeLa and HepG2 cells were seeded into 96-well plates at a density of 5×10^5 cells per well. After 24 h of culture, the cells were incubated with OVA, UiO-AM and UiO-OVA at various concentrations for 48 h in DMEM containing 10% FBS. Relative cell viability was measured by standard MTT assay and the average value of the three measurements was taken.

Cell Imaging

RAW264.7 cells were seeded into 36 mm cell dish in a 24-well plate and cultured for 24 h. Then the cells were incubated with UiO-OVA-FITC for 4 h at $37 \text{ }^{\circ}\text{C}$. The cells were then washed several times with PBS. Finally the imaging was captured using a Cytation™ 5 Cell Imaging Multi-Mode Reader.

OVA release of UiO-OVA

The complex UiO-OVA was re-suspended in 10 mL of Phosphate Buffered Saline (0.01M, pH 7.4). And then the re-suspended solution was incubated at 37°C with constant speed oscillation. Then, the solution is centrifuged at different time intervals and the content of the OVA was obtained from the supernatant solution using Bradford Protein Assay Kit (Beyotime, China).

Animal immunization

The Institutional Animal Care and Use Committee of the Changchun Institute of Applied Chemistry, Chinese Academy of Sciences approved this experiment. Twenty Kunming mice were supplied by the Medical Department of Jilin University (Changchun, China). The mice at an average age of 6-7 weeks were housed in a temperature-controlled room (25 ± 2 °C) with 12 h dark-light cycles and provided with food and water. Kunming mice were immunized with 25 µg of OVA, 25 µg of UiO-AM and 25 µg of UiO-OVA three times on days 0, 7, and 14, and sera were collected on day 21. Mice were immunized with various equivalent dose samples.

Serum antibody analysis

After one week following the final immunization, mice were sacrificed. Blood was collected and OVA specific antibody in sera was determined using ELISA. Micro titer plates by incubation of 100 µL of OVA antigen ($20 \mu\text{g mL}^{-1}$) in coating buffer (PBS, pH 7.2) for 4 °C overnight. The wells were blocked with 200 µL of blocking buffer (TBS containing 1% BSA and 0.1% tween-20, pH 7.4) at 37 °C for 2 h. After extensive washing with TBST (TBS containing 0.1% tween-20, pH 7.4), 20 dilutions of mouse serum in buffer (TBS containing 0.1% BSA and 0.1% tween-20, pH 7.4) was added into wells and then incubated for 1 h at 37 °C. After washing with TBST three times, HRP-labeled anti-mouse IgG antibody (Beyotime Institute of Biotechnology) was added, and then incubated for another 1 h at 37 °C. After washed three times, ELISA plate was incubated with TMB. After 10 min incubation at room

temperature, the color development was stopped by adding 50 μ L of Stop Solution (2 M H₂SO₄) and optical absorption was measured at 490 nm.

C3a Detection

A C3a sandwich enzyme linked immune sorbent assay (ELISA) was performed to measure complement activation in mice serum. Serum was incubated at 1:1 (100 μ L total volume) with OVA, UiO-AM or UiO-OVA at 37 °C for 30 min. The C3a content were measured by Mouse Complement Component 3a (C3a) ELISA Kit (Shang Hai Lengton Bioscience Co.,LTD). Absorbance was measured at 450 nm with a fluorescent microplate reader (Cytation™ 5 Cell Imaging Multi-Mode Reader).

The Effect of UiO-OVA on Lymphocyte Viability

After one week following the final immunization, mice were sacrificed and lymphocytes were harvested. The lymphocytes were treated with soluble 50 μ g of OVA, 50 μ g of UiO-AM (in terms of OVA), or 50 μ g of UiO-OVA at 37 oC for 60 h. The cells were analyzed by WST-1 assay.

The Effect of UiO-OVA on Splenocytes Proliferation

Lymphocyte proliferation was evaluated using CFDA staining. At 7 d after the final immunization, splenocytes were labeled with CFSE, and then treated with soluble 50 μ g of OVA, 50 μ g of UiO-AM (in terms of OVA) and 50 μ g of UiO-OVA at 37 oC for 66 h, respectively. The cells were harvested and analyzed by FACS.

Activation of CD4+ T and CD8+ T Cell

Lymphocyte Cells were seeded into 6-well plates at a density of 1×10^6 cells/well before they were incubated with 50 μ g of OVA, 50 μ g of UiO-AM (in terms of OVA), or 50 μ g of UiO-OVA for 66 h. Then the cells were stained with anti-CD3e-PE, anti-CD8a-FITC and anti-CD4-FITC antibodies (all from BD Biosciences).The stained cells were analyzed using FACS analysis.

Levels of IFN- γ , TNF- α and IL-12

To evaluate the levels of IFN- γ , TNF- α and IL-12 in mice serum, immunized mice were sacrificed. Serum was collected and the levels of IFN- γ , TNF- α and IL-12 in serum were quantified using ELISA kit according to the protocol of the Mouse cytokine kit (Boster, China).

Hemocompatibility Examination in vitro

The 10 μ L of fresh whole blood obtained from mice mixed with 10 μ L of OVA, UiO-AM, or UiO-OVA suspended in 0.9% stroke-physiological saline solution. The mixture was incubated at 37 °C with constant shaking. At timed intervals of 0.5, 1, 2, 4 and 6 h, one drop of the solution were sampled for the RBC morphology observation using Cytation™ 5 Cell Imaging Multi-Mode Reader.

Histopathological Examination

The injection site (skin and underlying tissue) was excised and immediately fixed in 4% paraformaldehyde. Tissues were paraffin embedded in paraffin, sliced and stained with hematoxylin and eosin. Stained samples were examined and recorded on Cytation™ 5 Cell Imaging Multi-Mode Reader.

Statistical Analysis

Data for cell viability, antibody and cytokines secretion assay were presented as mean \pm SD and were statistically analyzed using the two-tailed Student's T test. The statistical analysis was performed by using Origin 8.0 software. Asterisks indicate significant differences (*P < 0.05, **P < 0.01).

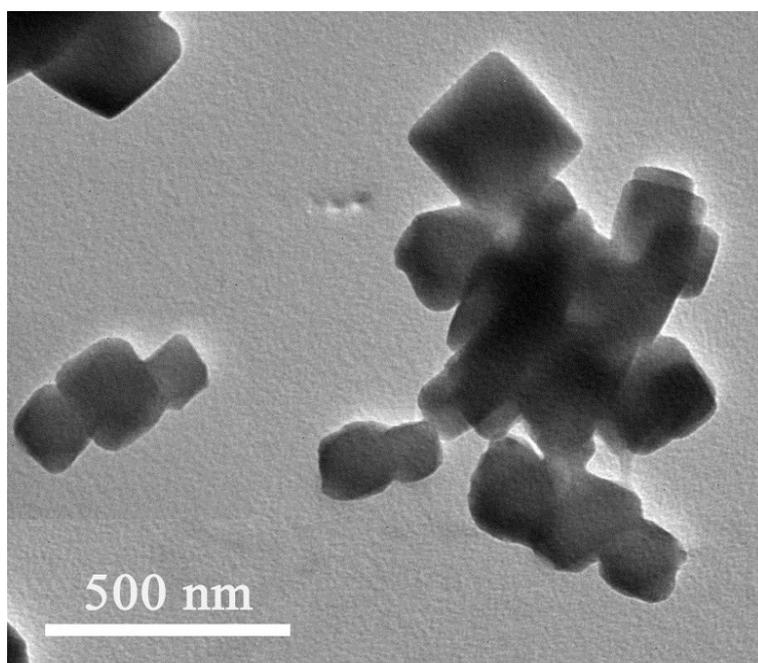


Figure S1. The TEM images of UiO-AM.

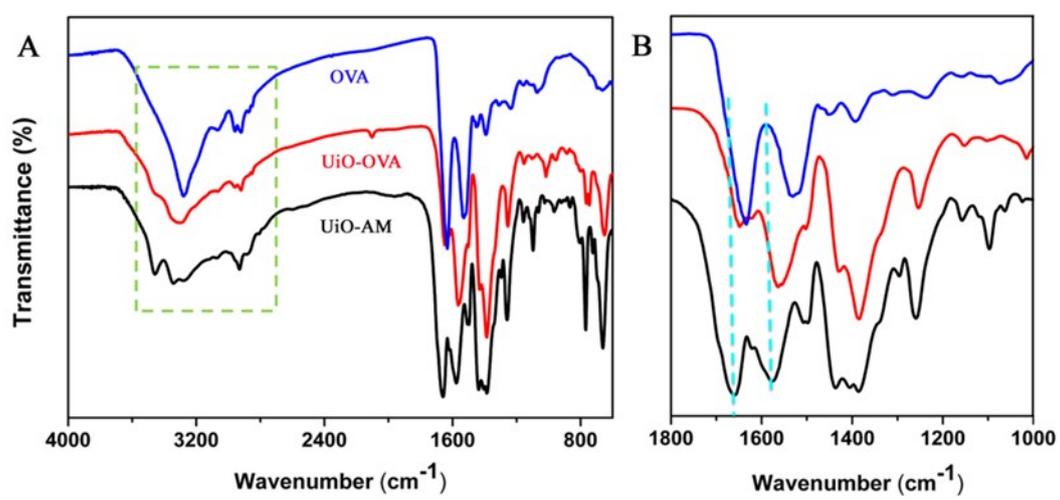


Figure S2. FTIR spectra of OVA, UiO-AM and UiO-OVA.

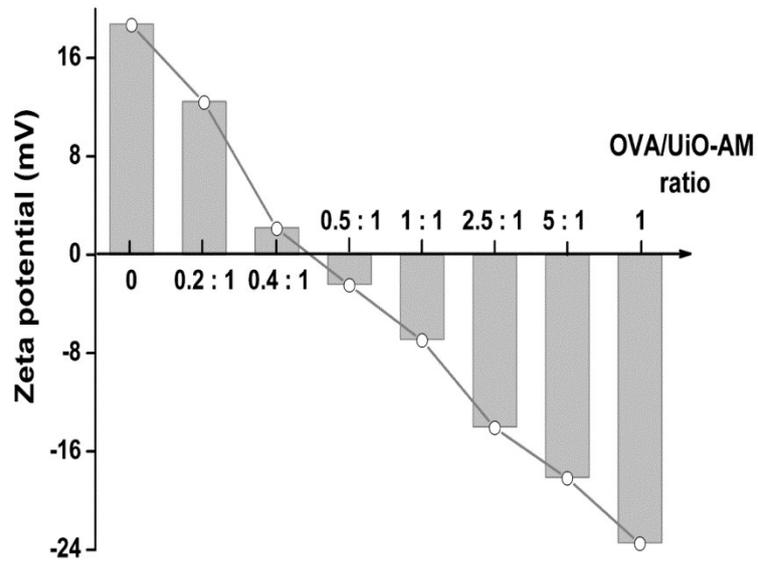


Figure S3. The Zeta potential of the OVA/UiO-AM ratio.

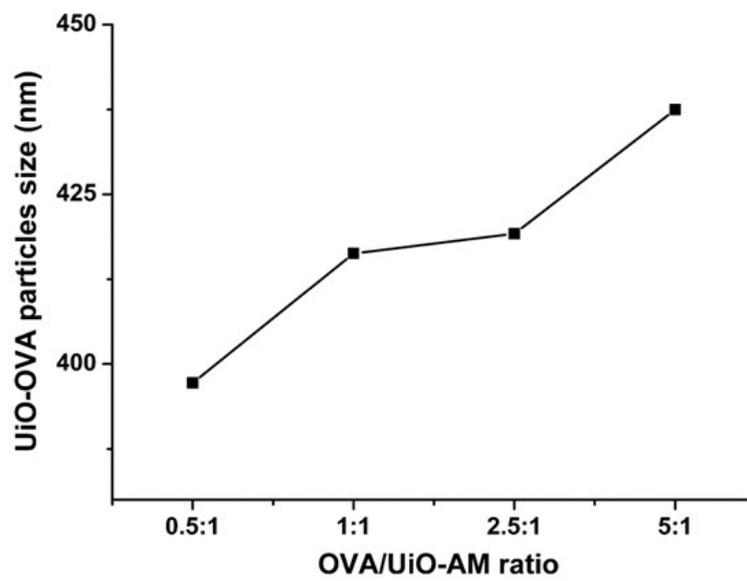


Figure S4. The diameters of UiO-OVA in different proportions with the OVA/UiO-AM ratio.

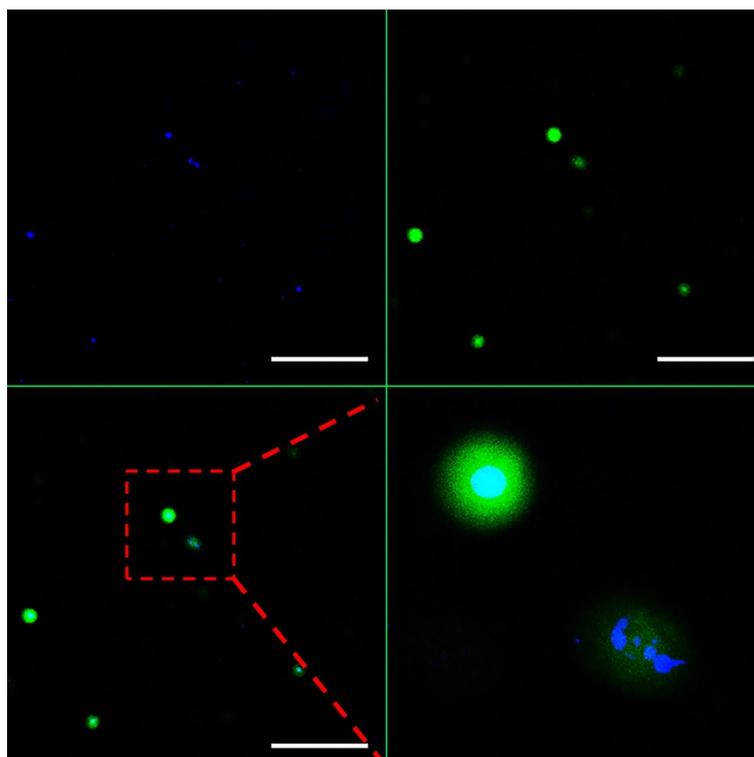


Figure S5. The CLSM images of UiO-OVA (Blue: UiO-AM, Green: OVA-FITC, Bar = 20 μm).

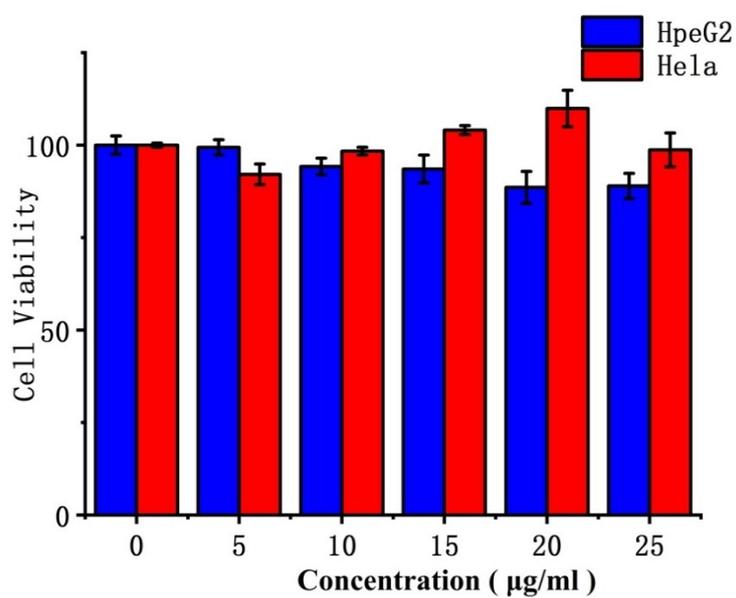


Figure S6. Cell viability of HpeG2 and HeLa cells in the presence of UiO-AM with various concentrations.

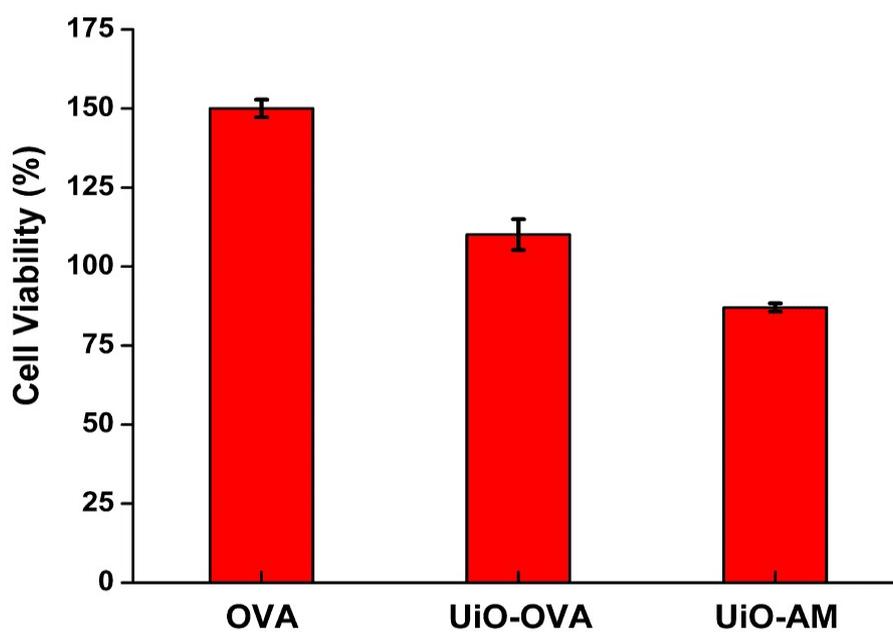


Figure S7. Cell viability of L929 cells in the presence of OVA, UiO-AM and UiO-OVA with $10 \mu\text{g mL}^{-1}$ OVA.

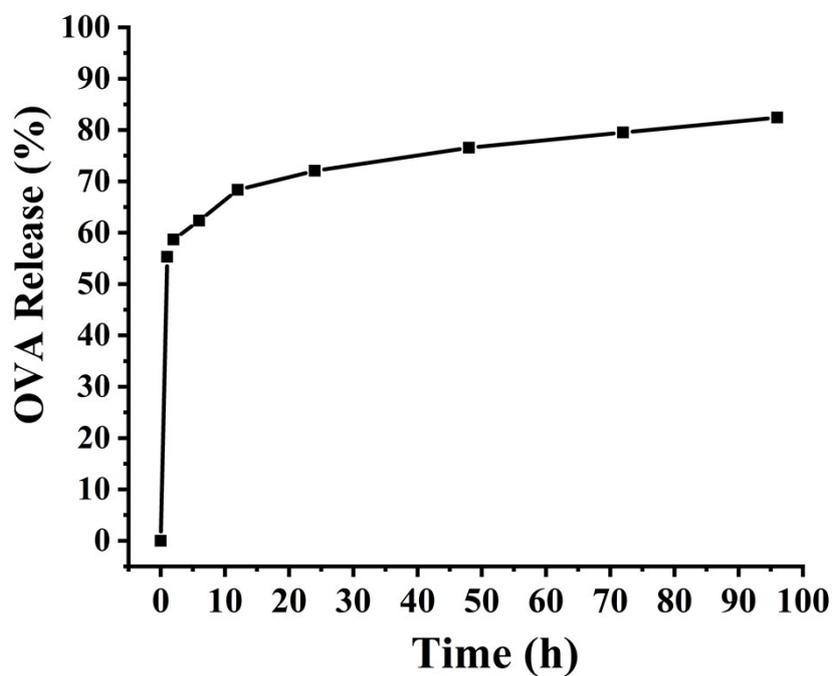


Figure S8. OVA release profiles of UiO-OVA in PBS (0.01M, pH 7.4).

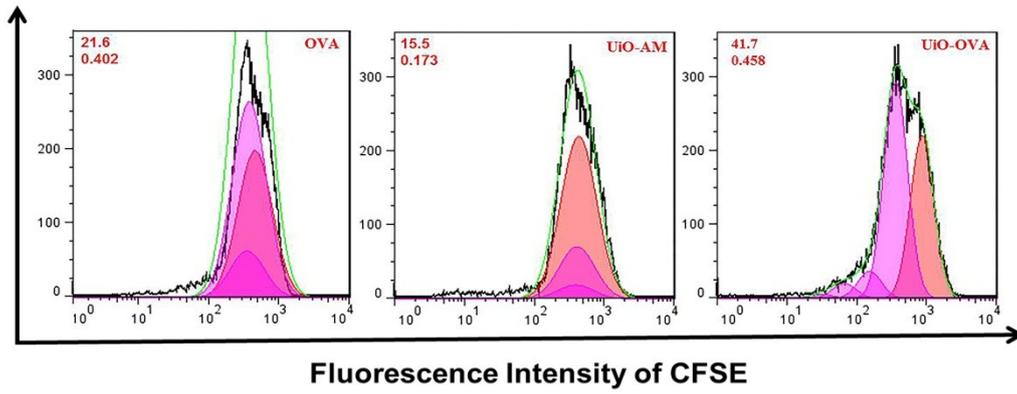


Figure S9. The lymphocytes proliferation capability was measured using FACS analysis after incubated with $50 \mu\text{g mL}^{-1}$ OVA for 66 h.

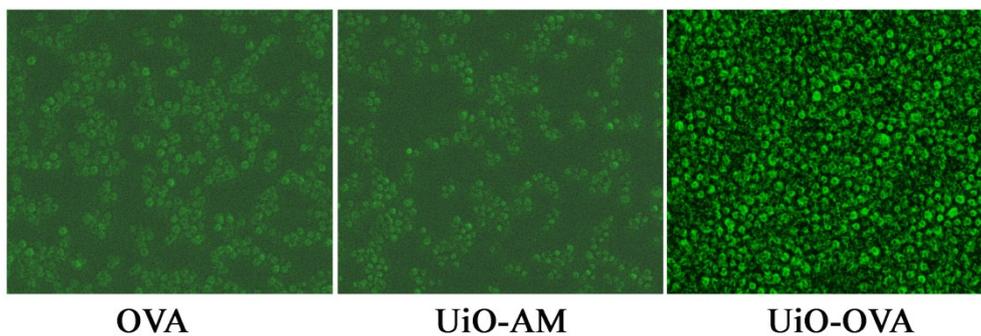


Figure S10. Fluorescence images of splenocytes with CFSE staining.

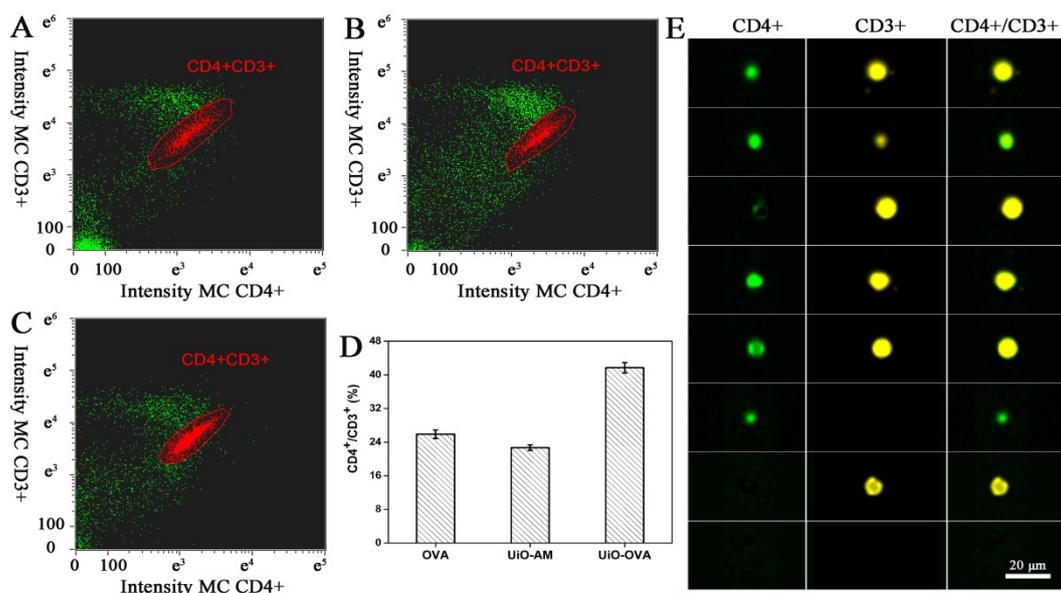


Figure S11. Mice immunized with UiO-OVA showed CD4⁺ T cell population in splenocytes ($n = 3$).

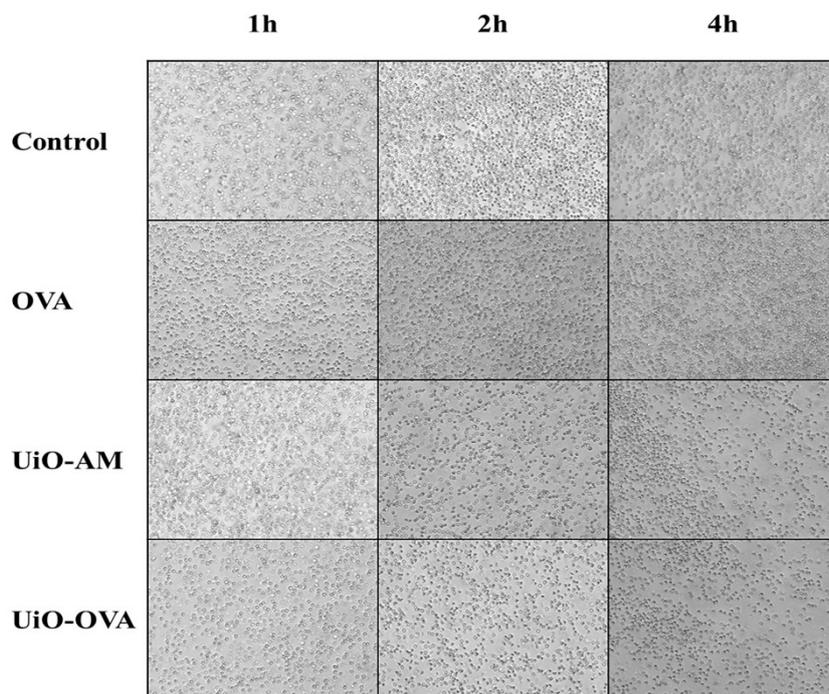


Figure S12. Morphology of RBC mixed with different solutions observed by microscope.

Reference

S1. L. Wang, W. Q. Wang, X. Zheng, Z Li, Z. Xie, *Chem-Eur J* 2017, 23, 1379.