Supporting Information

MnO_2 nanoparticles as a minimalist multimode vaccine adjuvant/delivery system to regulate antigen presenting cells for tumor immunotherapy

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Measurement

The Synthetic MnO_2 nanoparticles were examined with an X-ray diffractor (Miniflex 600, Rigaku, Japan). The ζ-potential, hydrodynamic size distribution of MnO_2 were measured through the dynamic light scattering instrument (Nano ZS, Malvern). The Mn concentration in MnO_2 was determined by an inductively coupled plasma-mass spectroscopy (ICP-MS, Thermo iCAP 7000 AERIES, USA).

Colloidal stability measurement

For the observation of colloid stabilization, 1mg/mL of MnO_2 were dispersed in DI water, PBS (10 mM, pH=7.4), saline, and RPMI-1640 culture medium respectively. Then the photos were taken to monitor the colloidal stability in 24 h. Furthermore, to test the stability of MnO_2 in water, PBS/pH7.4 solution, saline and 1640 medium solution, the particle size and zeta potential of the MnO_2 nanoparticles were measured by DLS at defined time points.

Slow release of Mn^{2+} from MnO_2 in vitro

The release of Mn^{2+} from the MnO_2 nanoparticles in vitro was investigated. The MnO_2 was incubated with phosphate buffer solution (pH5.0 or 7.4), and the solution was centrifuged (10000 rpm, 20 min) at different time points. Then, the Mn^{2+} in the supernatant was detected by ICP-MS. Then the photos were taken to monitor the stability in 7 days. Moreover, the Mn^{2+} release from the MnO_2 nanoparticles (in PBS/pH7.4 or 5.0) were detected by ICP-MS (the aqueous solution group is the control).

In vitro cytotoxicity analysis

The in vitro cytotoxicity of MnO_2 nanoparticles was detected by CCK-8 assay. Firstly, DC2.4, B16 and 4T1 cells were seeded in a 96-well plate at density of 1×10^4 cells/well, respectively. After overnight incubation, the original medium was replaced with fresh complete medium containing MnO_2 with different concentration. Subsequently, the cells were incubated at 37°C for 24 h. After 24 h, the cells were washed with PBS, then, 100 μL of fresh medium containing 10% CCK-8 was added to each well. The cells were incubated again for 20 minutes. Finally, the absorbance at 450 nm of each well was detected by a microplate reader (Cytation5, Biotek, USA).

Detection of intracellular reactive oxygen species (ROS)

To detect intracellular ROS in vitro, briefly, DC2.4 cells were seeded into 6-well plates at a density of 1×10^5 cells per well and incubated at 37℃ overnight. Then, cells were treated with the MnO_2 nanoparticles at different concentration and PBS for 24 h. After 24 h, 2', 7'-dichlorofluorescin diacetate (DCFH-DA) was added to each well and incubated for 30 min at 37℃.Finally, the fluorescence was detected by fluorescence microscope.

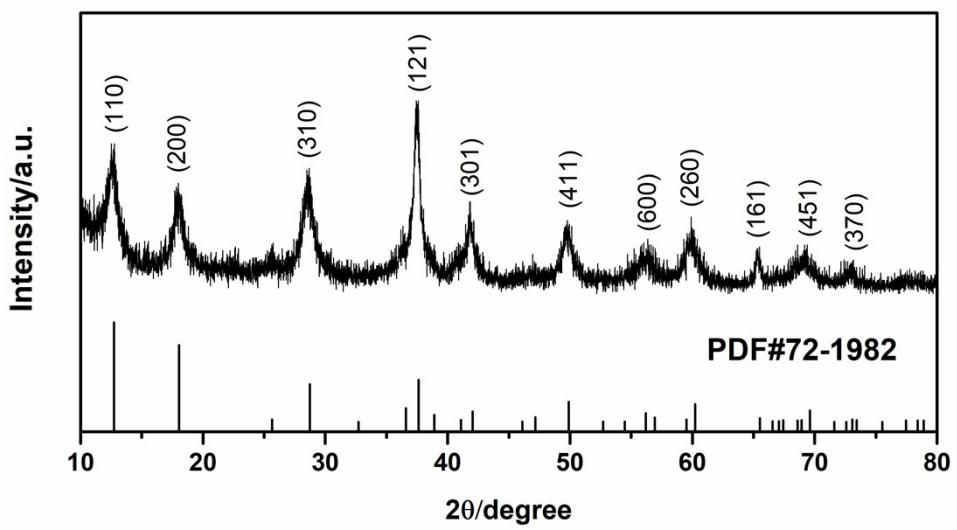

Figure S1. XRD spectrum of MnO_2

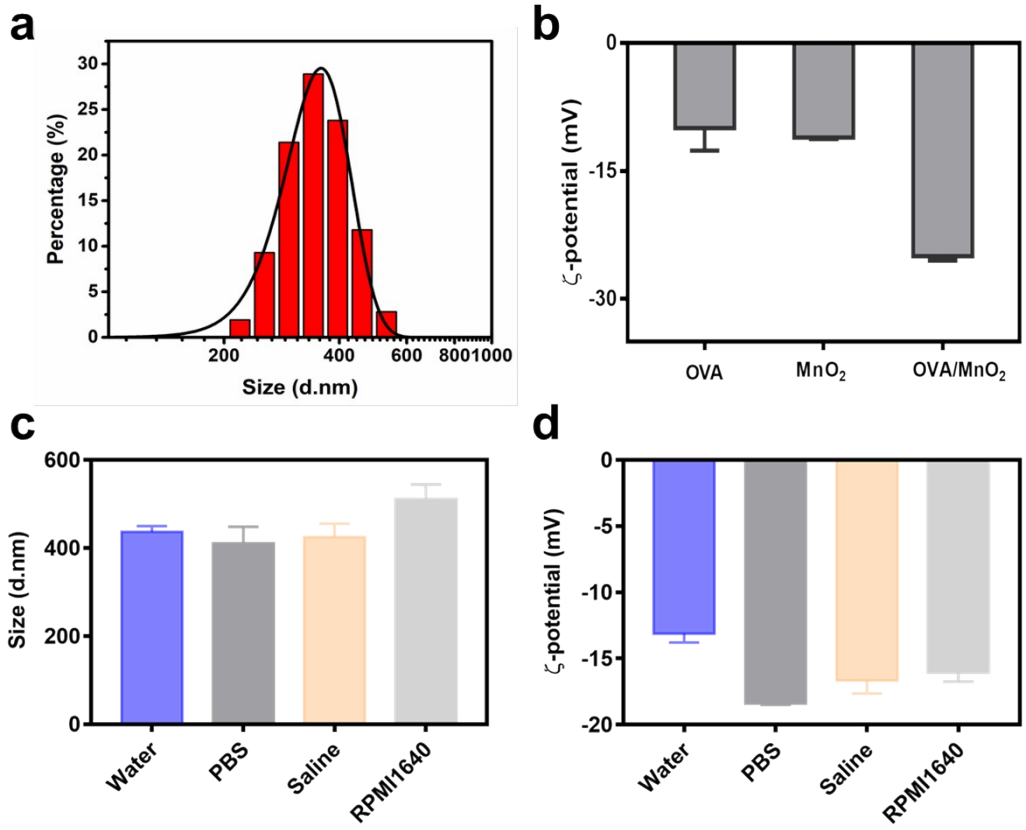

Figure S2. (a) The size distribution of the MnO_2 nanoparticles. (b) Zeta potentials of OVA, MnO_2, OVA/MnO_2 measured by dynamic light scattering (DLS). The average zeta potentials of OVA, MnO_2 and OVA/MnO_2 are -9.99, -11.1 and -25 mV, respectively. Compared with MnO_2, the increase of OVA/MnO_2 is attributed to electronegativity of OVA, indicating successful OVA coating. (c) Particle size and zeta potential (e) of the MnO_2 nanoparticles in various solutions, including water, PBS, saline, RPMI1640 basal medium.

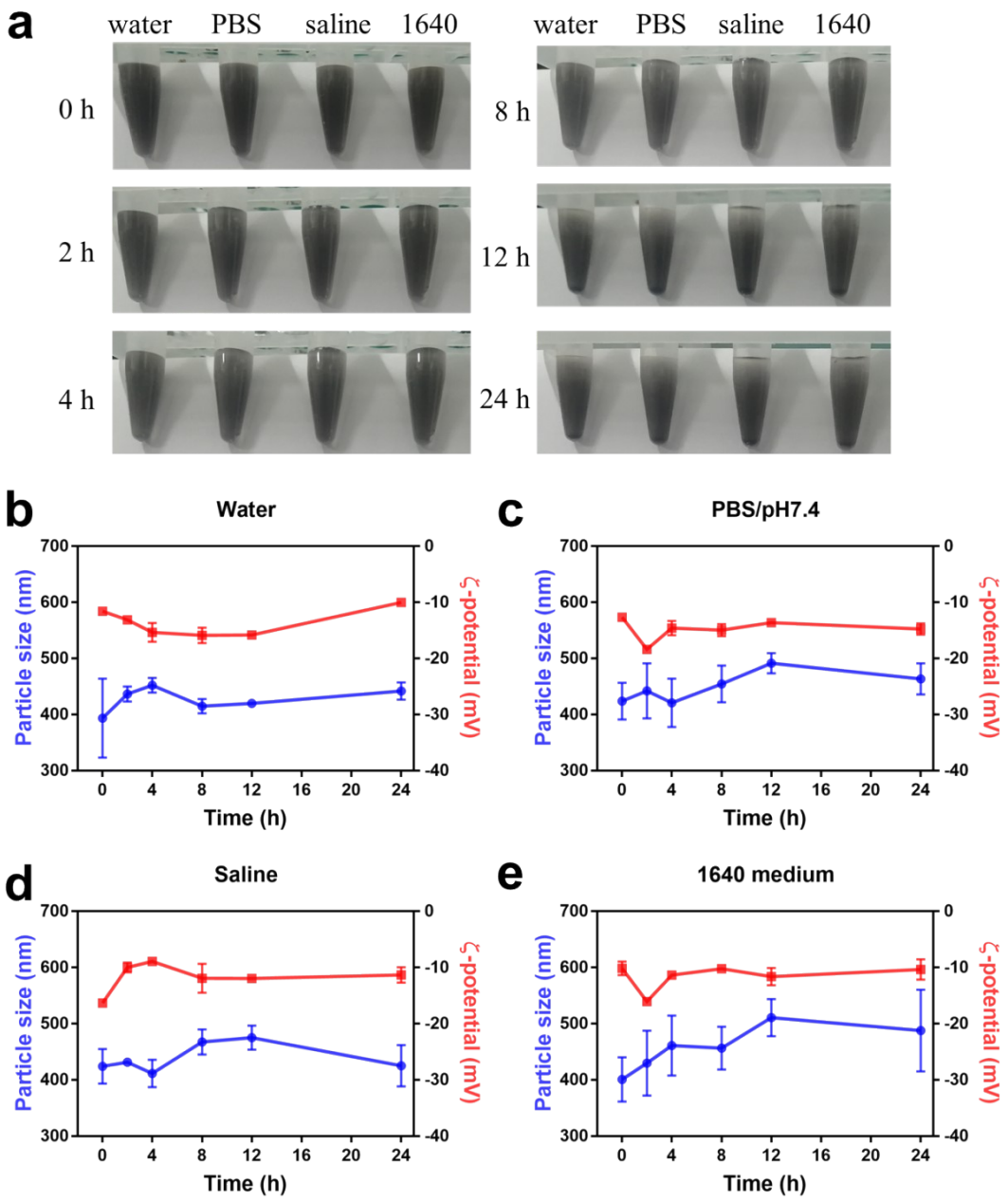

Figure S3. (a) Colloidal stability. (b-e) Size distribution and zeta potential changes of the MnO_2 namoparticels in 24 h in (b) water; (c) PBS/pH7.4; (d) saline and (e) 1640 medium.

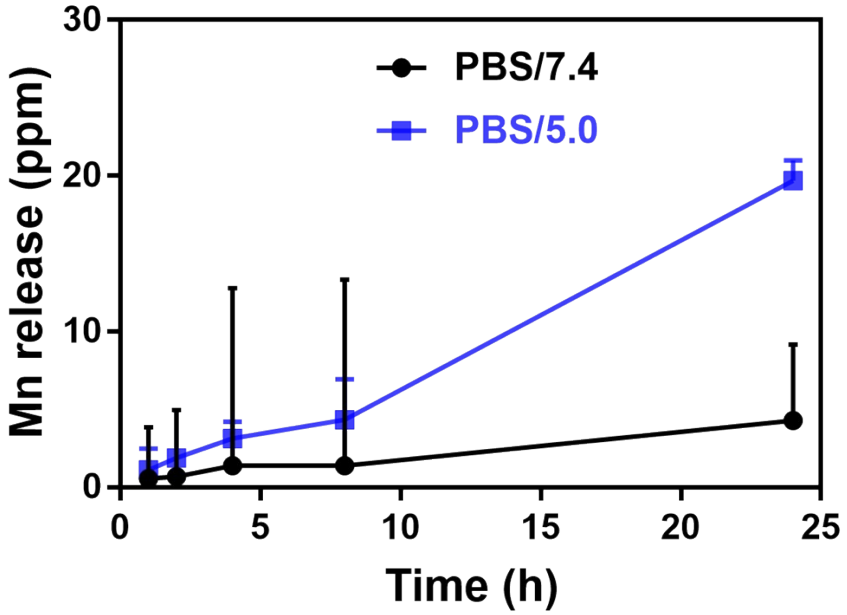

Figure S4. Mn ions release from the MnO_2 suspension (in phosphate buffer solution of pH7.4 or pH5.0) within 24 h.

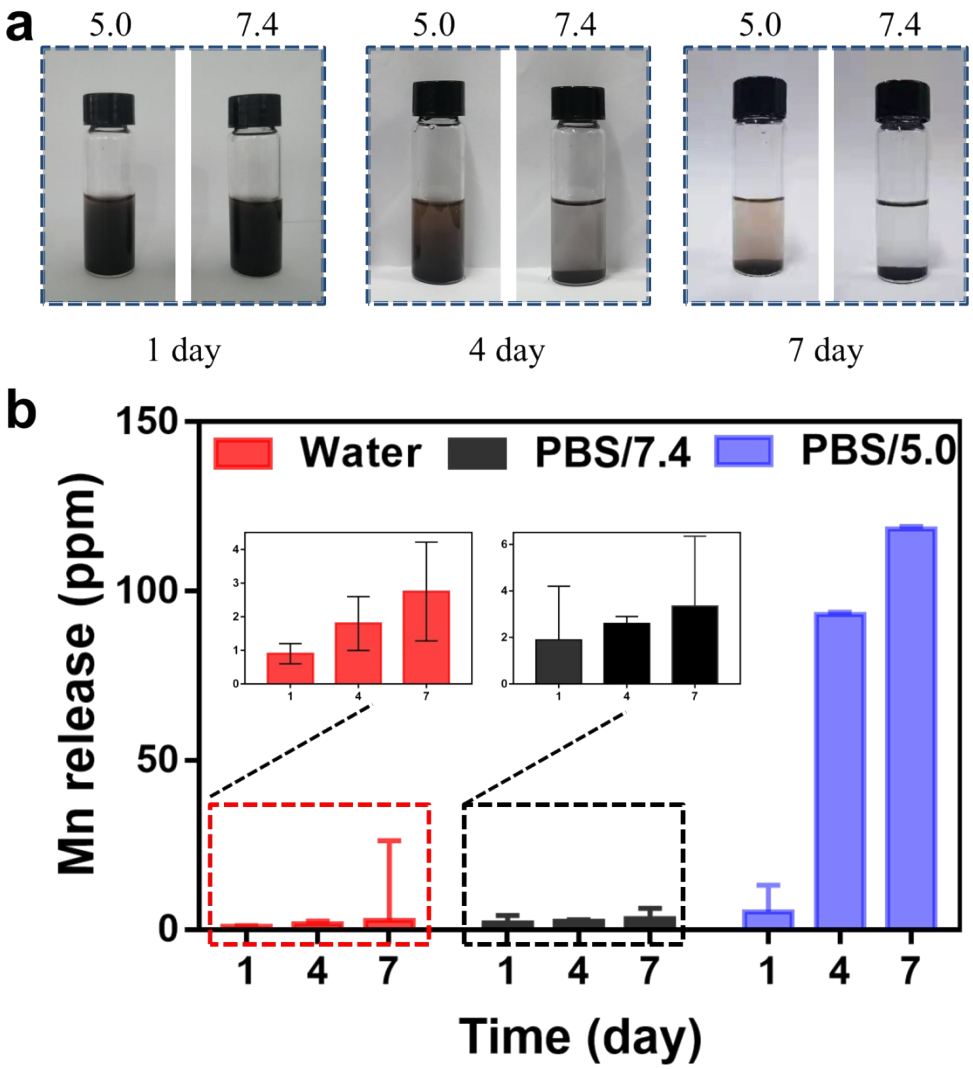

Figure S5. (a) Stability observation of the MnO_2 at different pH values (5.0 and 7.4) over time. (b) Mn ions release from the MnO_2 aqueous solution within 7 days.

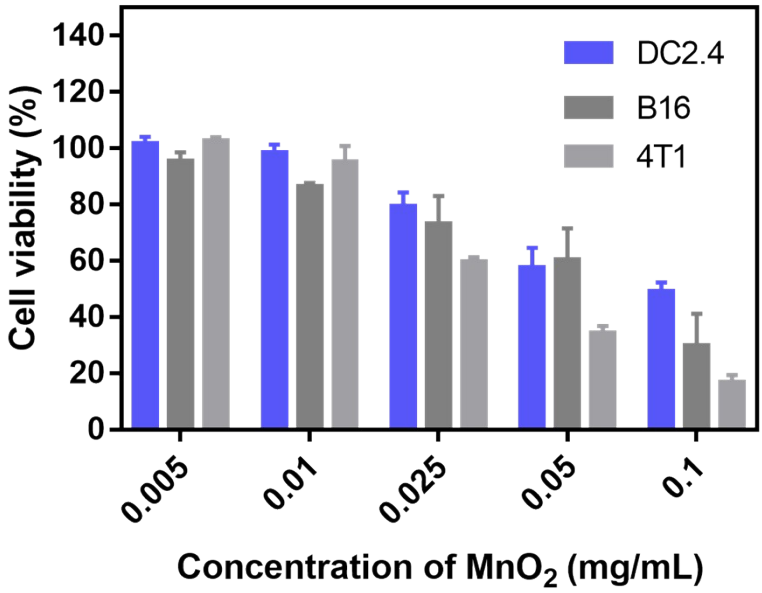

Figure S6. In vitro cytotoxicity of the MnO_2 nanoparticles in DC2.4, B16 and 4T1 cells.

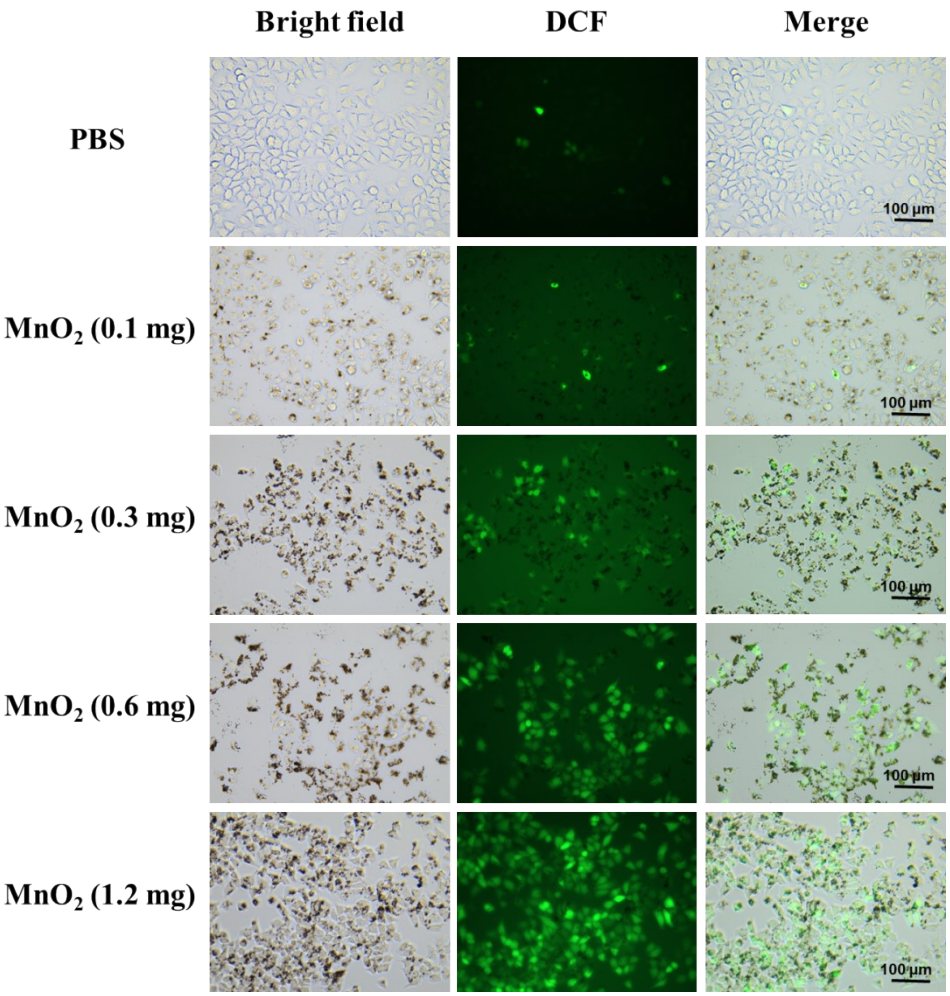

Figure S7. Intracellular HO· detections using DCFH-DA as a probe in different samples (PBS and MnO_2). Each series can be classified into bright field, green fluorescence from DCF and merge, respectively. The enhanced green fluorescence from MnO_2-treated group was observed in comparison with the control group.

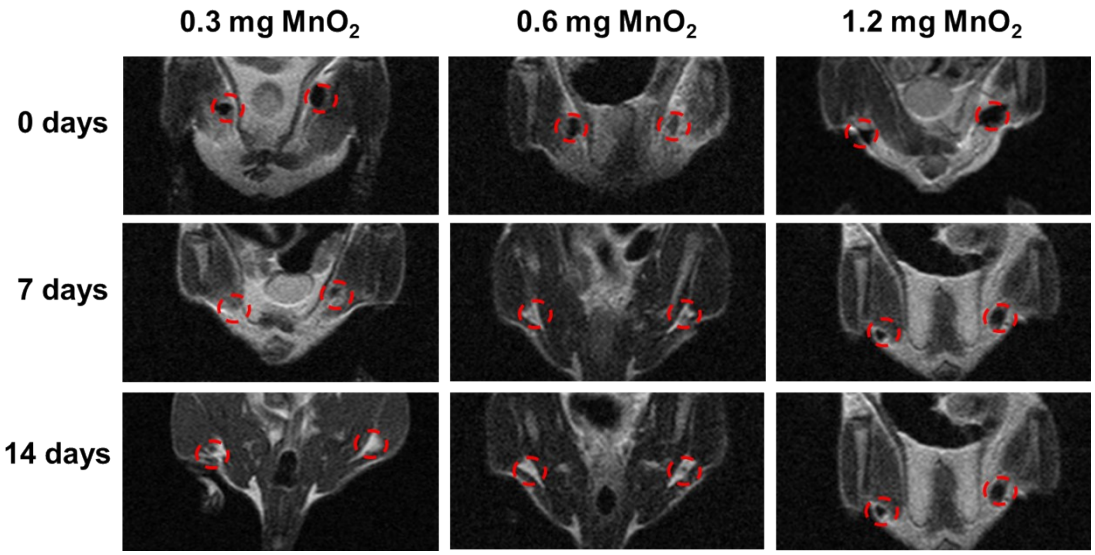

Figure S8. MRI of materials at different time points at the injection site of mice.

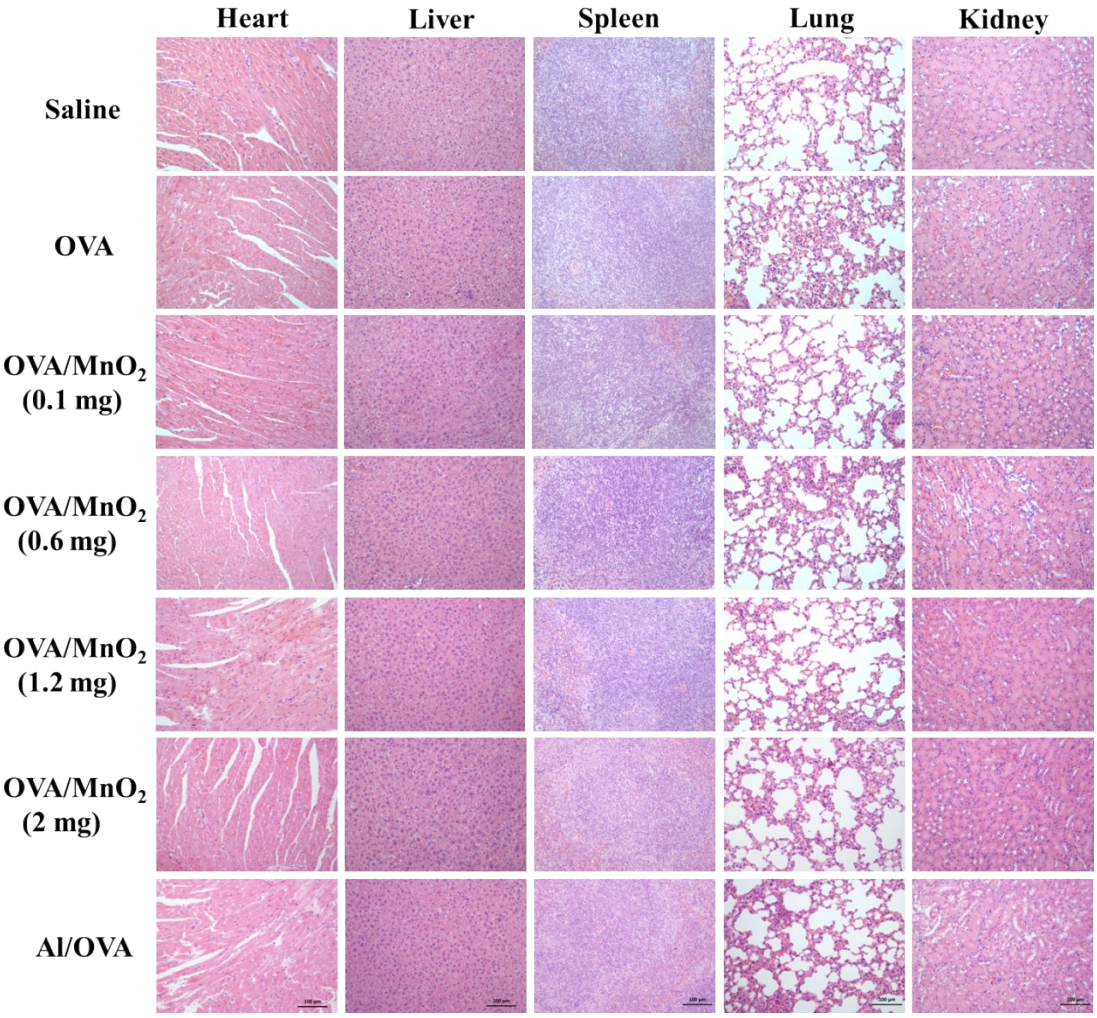

Figure S9. Evaluation of the biological safety of key organs (heart, liver, spleen, lung and kidney) after subcutaneous immunization with different vaccine formulations. Scale bar, 100 μm.

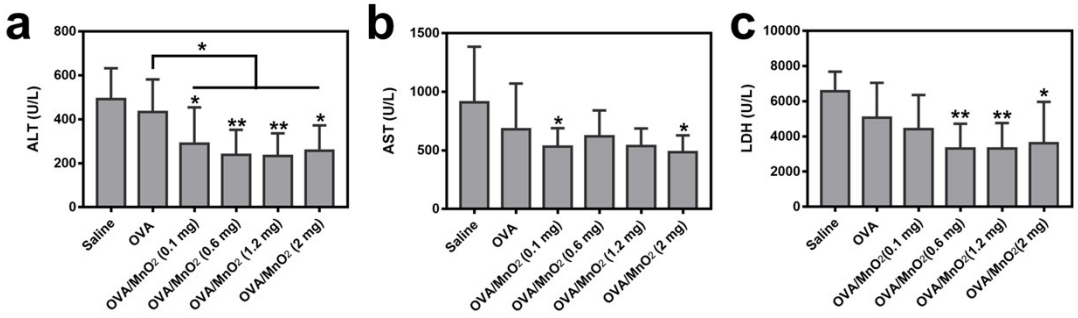

Figure S10. Serum of alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) in mice measured at 23 d during the course of treatment. (*P＜0.05, **P<0.01; n=5).