Supporting Information

Materials and Methods

Chemicals and Materials.

Purified anti-mouse TNF-α, biotin conjugated anti-mouse TNF-α cocktail, mouse TNF-α recombinant protein, anti-mouse IFN-γ, biotin anti-mouse IFN-γ, mouse IFN-γ recombinant protein, anti-Mouse IgG biotin, anti-Mouse IgG2a biotin, Anti-Mouse IgG1 biotin, and avidin-HRP were purchased from eBioscience. NF-κB p65 primary antibodies, Cy3 labelled second antibody from Beijing Biosynthesis Biotechnology Co., LTD were used for immunohistochemistry (IHC). OPD (o-phenylenediamine) substrate was obtained from DingGuo. Thiazolyl blue tetrazolium bromide (MTT), 4-carboxyphenylboronic acid (CBA), LPS (*E.coli* 055:B5), cetyltrimethylammonium tosylate (CTATos), tetraethyl-orthosilicate (TEOS), Rhodamine B isothiocyanate (RITC), ovalbumin (OVA) were purchased from Sigma-Aldrich (USA). Dulbecco's modified Eagle's medium (DMEM) and fetal Bovine Serum (FBS) were purchased from Invitrogen. N-cetyltrimethylammonium bromide (CTAB) was obtained from Alfa Aesar. Triethanolamine and fluorescein isothiocyanate (FITC) was purchased from Aladdin. Nanopure water (18.2 MΩ·cm; Millpore Co., USA) was used in all experiments and to prepare all buffers.

Measurements and characterizations

A field emission scanning electron microscope (FESEM, S4800, Hitachi) was applied to determine the morphology and composition of the as-prepared samples. TEM was performed on a JEOL 1011 transmission electron microscope at an accelerating voltage of 200 kV. N_2 adsorption-desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. FT-IR analyses were carried out on a Bruker Vertex 70 FT-IR Spectrometer. Thermogravimetric analyses were carried out on a PerkinElmer Pyris Diamond TG/DTA Analyzer, using an oxidant atmosphere (Air) with a heating program consisting of a dynamic segment.

Synthesis of mesoporous silica nanoparticles (MSNs) with large pores

A typical synthesis of MSNs was performed according to the method reported

previously. A mixture of 1.92 g of CTATos and 0.347 g of triethanolamine were added into100 mL of deionized water and stirred at 80°C for 1 hour. Then 14.58 g of TEOS was quickly added into the surfactant solution. The mixture was stirred at 80°Cfor another 2 hours. The synthesized MSNs were filtered, washed, and dried in the oven at 100°C for 20 hours. Finally, the materials were calcined at 600°Cfor more than 6 h to remove the surfactant.

Synthesis of fluorescein isothiocyanate (FITC) and Rhodamine B isothiocyanate (RITC)-labeled nanoparticles (MSN@FITC and MSN@RITC)

As for FITC or RITC-labeled nanoparticles, 1 mg FITC or RITC was reacted with 22 μL of APTES in 1 mL ethanol for 2 h in the dark. Then, 20 μL was added into MSN solution (2 mg/ml, 10 mL). Finally, ammonia (50 μL) was added and the mixture was stirred at room temperature for 24 h, followed by centrifugation and washing with water.

Chemical modification of the MSN surface (MSN-CBA)

To get amino-modified nanopatices, MSN (1.00 g) was refluxed for 24 h in 100 mL of anhydrous toluene with 1 mL of APTES. 0.15 g CBA was reacted with 0.1 g NHS and 0.2 g EDC in 5 mL DMSO, stirring at room temperature for 6 h to activate the carboxylic group of CBA. 400 mg MSN-NH_2 was dispersed in 20 mL dimethyl sulfoxide (DMSO) and were added to the above solution. The mixture was stirred at room temperature for another 24 h, followed by centrifugation and washing with DMSO, and water.

Preparation of detoxified LPS (SP-LPS)

SP-LPS were prepared by the method of previous reports. Briefly, 10 mg of LPS was mixed with 100 mg of phthalic anhydride for 36 h in 2 mL of a 1:1 mixture of pyridine and formamide. After the completion of the reaction, it was dialyzed using a 30 kDa cutoff membrane, first against water, then against 0.1 M sodium bicarbonate and finally against water. The dialyzed sample was then lyophilized to obtain dry powdered SP-LPS.

The typical OVA loading experiment (MSN-OVA)

OVA loading onto MSNs was done by mixing 2 mg/mL OVA with MSNs (2 mg/mL)

in different pH of PBS buffer (pH= 5.0, 7.5, 8.0) overnight. For the OVA loading saturation experiment, different concentrations of OVA were mixed with MSNs (0.2 mg/mL) in PBS (pH = 5.0) overnight. The supernatant was collected by centrifugation at 8000 rpm for 8 min. The supernatant and stock solution was analyzed by UV–Vis spectrophotometry (Cary 300, Varian Inc., Palo Alto, CA) at 280 nm, respectively.

Preparation of MSN-OVA-SP-LPS complexes

The MSN-CBA-OVA (20 mg) was incubated with SP-LPS in PBS solution. The mixture was stirred for 24 h, following by filtration and washing extensively with PBS to remove physisorbed SP-LPS from the exterior surface of the material.

Conjugation of fluorescein isothiocyanate (FITC) to OVA (OVA-FITC)

Twenty milligrams FITC was dissolved in 10 ml of carbonate buffer (220 mM, pH 9.5) and 100 mg OVA was added. The mixture was gently stirred in the dark at 4 °C for 18 h. Unbound FITC were removed by dialysis (MWCO 10000). The resulting OVA-FITC solution was freeze-dried.

Cell culture

The mouse leukemic monocyte macrophage cell line (RAW264.7 cell line) was purchased from Cell Bank of Chinese Academy of Sciences (Shanghai). The murine macrophage-like RAW264.7 cells were grown at 37 °C in an atmosphere of 5% (v/v) CO_2 in air., in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated FBS, 1.5 g/L $NaHCO_3$, 100 units/mL penicillin, 100 mg/ml streptomycin, 4.5 g/L glucose and 4 mM/L glutamine. The media was changed every three days, and the cells were digested by trypsin and resuspended in fresh complete medium before plating.

Cytotoxicity assays

MTT assays were used to probe cellular viability. RAW264.7 cells were seeded at a density of 5000 cells/well (100 μL total volume/well) in 96-well assay plates. After 24 h incubation, the as-prepared MSN-SP-LPS complexes, at the indicated concentrations, were added for further incubation of 48 h. To determine toxicity, 10 μL of MTT solution (BBI) was added to each well of the microtiter plate and the plate was incubated in the CO_2 incubator for an additional 4 h. Then the cells were lysed by

the addition of 100 μL of DMSO. Absorbance values of formazan were determined with Bio-Rad model-680 microplate reader at 490 nm (corrected for background absorbance at 630 nm). Six replicates were done for each treatment group.

Measurement of NF-κB activity in RAW264.7 cells

Briefly, cells were harvested and washed twice with PBS, and fixed in 4% paraformaldehyde for 20 min. Cells were washed for 5 min with PBS containing 0.1% Triton X-100 and incubated with mouse polyclonal antibody against p65 NF-κB for 1 h at 37 °C in the dark. The cells were then labeled with Cy3-conjugated rabbit anti-mouse IgG monoclonal antibody for 30 min in the dark before staining with DAPI.

Uptake studies of MSN-SP-LPS

Uptake of nanoparticles by RAW264.7 cells was assessed by confocal microscopy and flow cytometry. RAW264.7 cells were incubated with MSN@RITC or MSN@RITC-SP-LPS at desired concentrations for 2 h, and were fixed in 4% paraformaldehyde. Cells were counterstained with DAPI (nuclear stain) and imaged with a confocal microscope or analyzed by flow cytometry.

Detection of Intracellular ROS

Intracellular ROS generation was measured using 2,7-dichlorofluorescein diacetate (DCFH); DCFH is a non-fluorescent compound that undergoes intracellular deacetylation, followed by ROS mediated oxidation to a fluorescent dichlorofluorescin (DCF). For the cell imaging test, the concentration of RAW264.7 cells was fixed at a density of 10^5 cells/well in 24-well assay plates. After various experimental treatments (Rosup, MSN@RITC-SP-LPS, MSN@RITC, SP-LPS), cells were incubated with DCFH for 30 min and intracellular ROS generation was monitored using an Olympus BX-51 optical equipped with a CCD camera.

OVA-Releasing Experiments

The release of OVA in vitro was monitored by fluorescence microscopy. RAW264.7 cells were incubated with MSN@RITC-OVA-FITC-SP-LPS for 1 h and 3 h before fluorescence microscopic monitoring.

Cytokine Assays

RAW264.7 cells were seeded on 24-well culture plates at a density of 5×10^5 cells/well. After 24 h incubation, cells were washed with 0.5 mL PBS before treatment with indicated conditions for 8 h (TNF-α). The supernatants were collected and stored at 80°C until use. The levels of TNF-α in the supernatants were determined by enzyme-linked immunosorbent assay (ELISA) using antibody pairs specific to these cytokines following protocols recommended by the manufacturer.

Histology analysis

For histology studies, mice were sacrificed 14 days after administration. The tissues (heart, spleen, liver, lung, and kidneys) were collected from above two groups (control and test groups), and fixed in 10% neutral buffered formalin. Then, the collected tissues were embedded in paraffin, sectioned (4 mm thick), as well as stained with hematoxylin and eosin (H&E). The histological sections were observed under an optical microscope.

Immunogenicity Study

Kunming mice of 4-6 weeks were purchased from Medical Experimental Animal Center of Jilin University (Changchun, China) and all animal procedures were in accord with the guidelines of the Institutional Animal Care and Use Committee. Mice were immunized with 10 μg/animal of OVA or OVA in nanoparticles, by the subcutaneous injection in a final volume of 0.1 mL. Mice were injected on days 0, 14, 28 and sera were collected on day 42.

Titer Measurements

Micro titer plates (Nunc, Roskilde, Denmark) were coated with OVA by incubation of 100 μL 20 μg/mL OVA in coating buffer (PBS, pH 7.2) for 24 h at 4 °C. To reduce aspecific binding, wells were blocked with 200 μL 1% (w/v) BSA in PBST (0.05% Tween-20) for 1 h at 37°C. After extensive washing with PBST, serial dilutions (1:100, 1:1000, 1:5000, 1:10000, 1:50000, 1:100000, 1:200000) of sera samples were added and sera sample (control group treated with PBS) was diluted to 1:100. After incubation for 1.5 h at 37 °C and washed three times, the antibody of Anti-Mouse IgG, IgG2a and IgG1 biotin antibody was diluted to 1/2000 and incubated (100 μL per well) for 1 h at 37°C. Then avidin-HRP was diluted to 1/500 and added into well. After

washed four times, 0.4 mg/mL o-phenylenediamine (4 mg o-phenylenediamine was dissolved in a buffer containing 4.86 mL 0.1 M critic acid and 5.14 mL 0.2 M Na_2HPO_4) with 30% H_2O_2 (0.5 μL/mL) was added to wells (100 μL per well) as substrate. After 30 min incubation at room temperature, the color development was stopped by adding 50 μL of Stop Solution (2 M H_2SO_4) and optical absorption was measured at 490nm. The end-point titer of IgG, IgG2a and IgG1 was determined by the reciprocal of maximal serum dilution that exceeded twice the SD above the mean control group OD. The individual antibody titers expressed as $[\log_2[\bar{x} \pm SD]]$, calculated as the reciprocal of maximal serum dilution.

Statistical Analysis

All data were expressed in this article as mean result ± standard deviation (SD). Data were analyzed for statistical significance using Student's test. $p<0.01$ was considered statistically significant, $p<0.005$ and $p<0.001$ was considered highly significant. All figures shown in this article were obtained from three independent experiments with similar results. The statistical analysis was performed by using Origin 8.0 software.

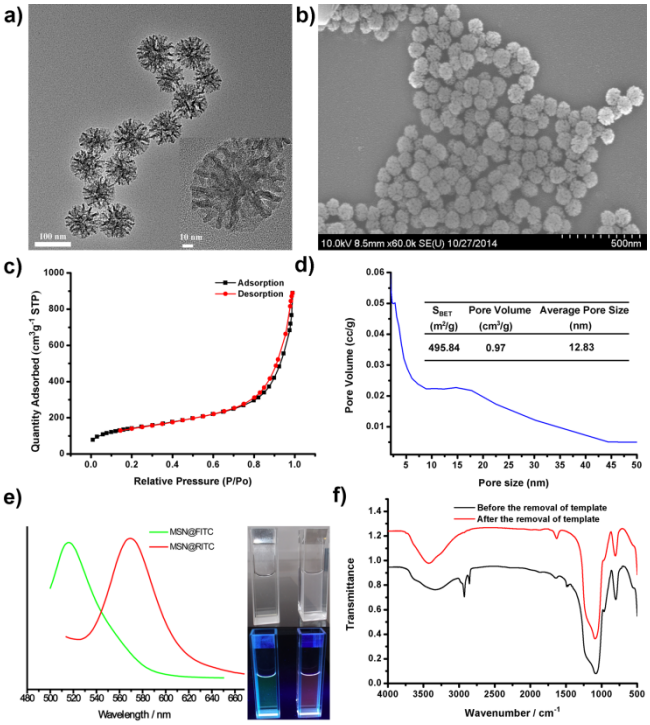

Fig. S1. TEM image (a), SEM image (b), N_2 adsorption–desorption isotherms (c) and pore parameters of MSNs with large pores. (e) Photoluminescence spectra of MSN@FITC (λ_{ex} =488 nm) and MSN@RITC (λ_{ex} = 520 nm). (f) FT-IR spectra of MSN with large pores before and after the removal of template. The complete extraction of the template was validated by FTIR spectroscopy with the absence of the characteristic C-H peak in the 3000-2800 cm^{-1}. The removal of template would ensure the biocompatibility of the large pore materials and the loading of antigens.

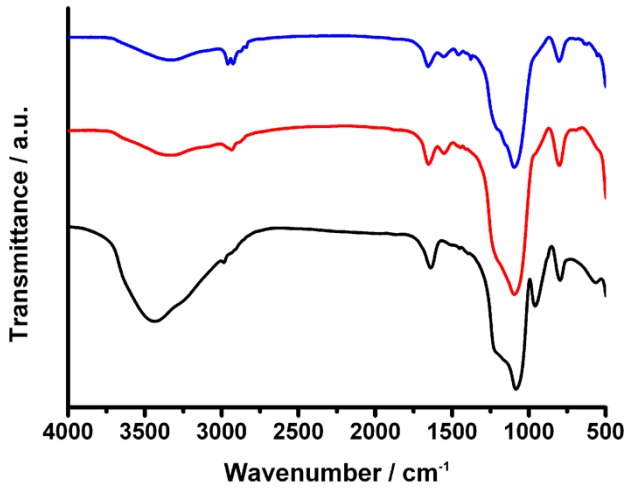

Fig. S2. The functional MSN was monitored by FTIR spectroscopy. MSN-NH_2

(black), MSN-CBA (red) and MSN-SP-LPS (blue). Successful grafting of CBA onto the mesoporous silica was validated by the appearance of the typical acylamide vibration peaks at 1562 cm^{-1}. The peak at 3060 cm^{-1} was characteristic signals of SP-LPS，confirming the existence of SP-LPS on the surface of MSN.

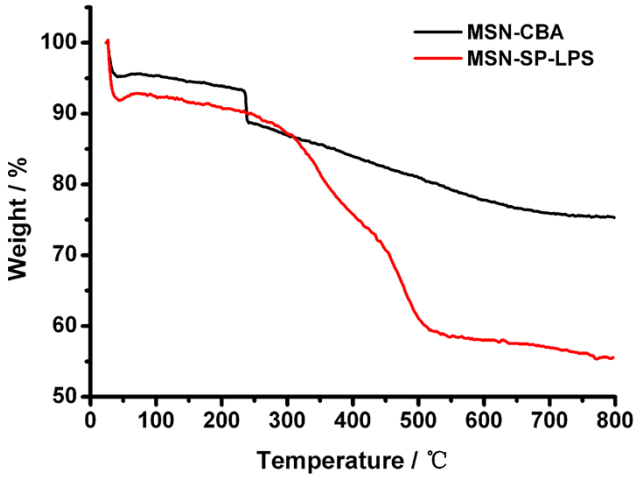

Fig. S3. Thermo gravimetric analysis of MSN-CBA and MSN-SP-LPS. A mass loss of 20% during heating to 800°C was evaluated to correspond to 0.2 mg SP-LPS per milligram of material.

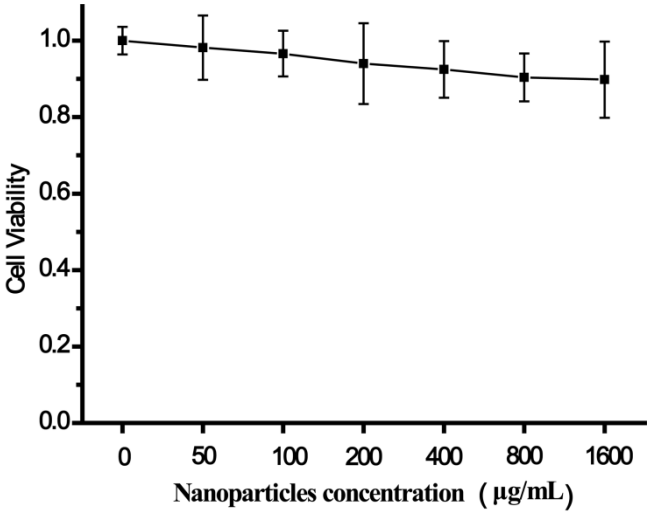

Fig. S4. Cell ability of RAW264.7 cells treated with MSN-SP-LPS at different concentration.

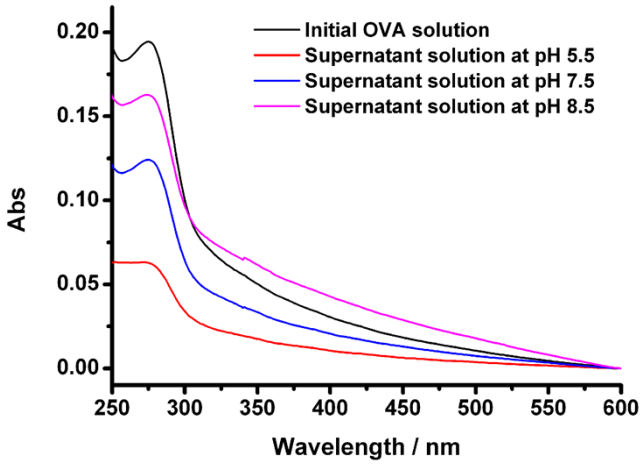

Fig. S5. OVA loading onto MSNs with large pores at different pH.

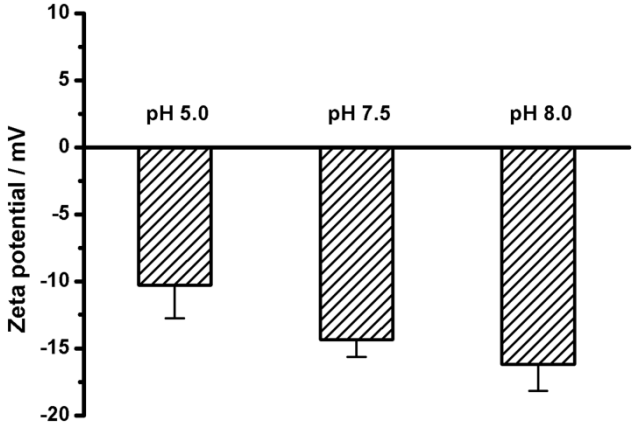

Fig. S6. Zeta potential of MSN at different pH.

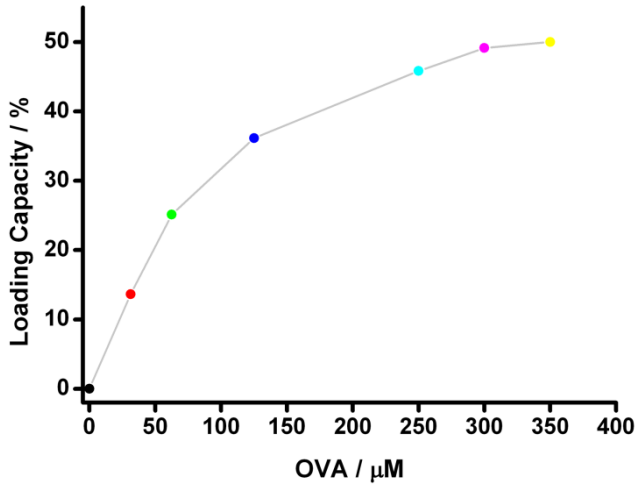

Fig. S7. Quantification of OVA loading at different OVA concentrations.

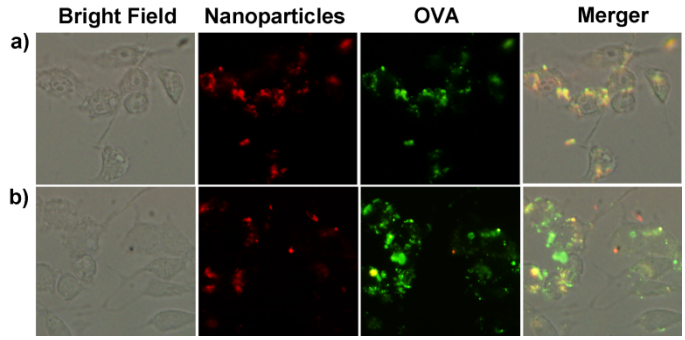

Fig. S8. After modified by SP-LPS, OVA-FITC-loaded MSN@RITC nanoparticles were incubated with RAW264.7 cells and the behavior of release was monitored by fluorescence microscope. Fluorescence microscope images of RAW264.7 cells incubated with MSN@RITC-OVA-FITC-SP-LPS for 1 h and 3 h.

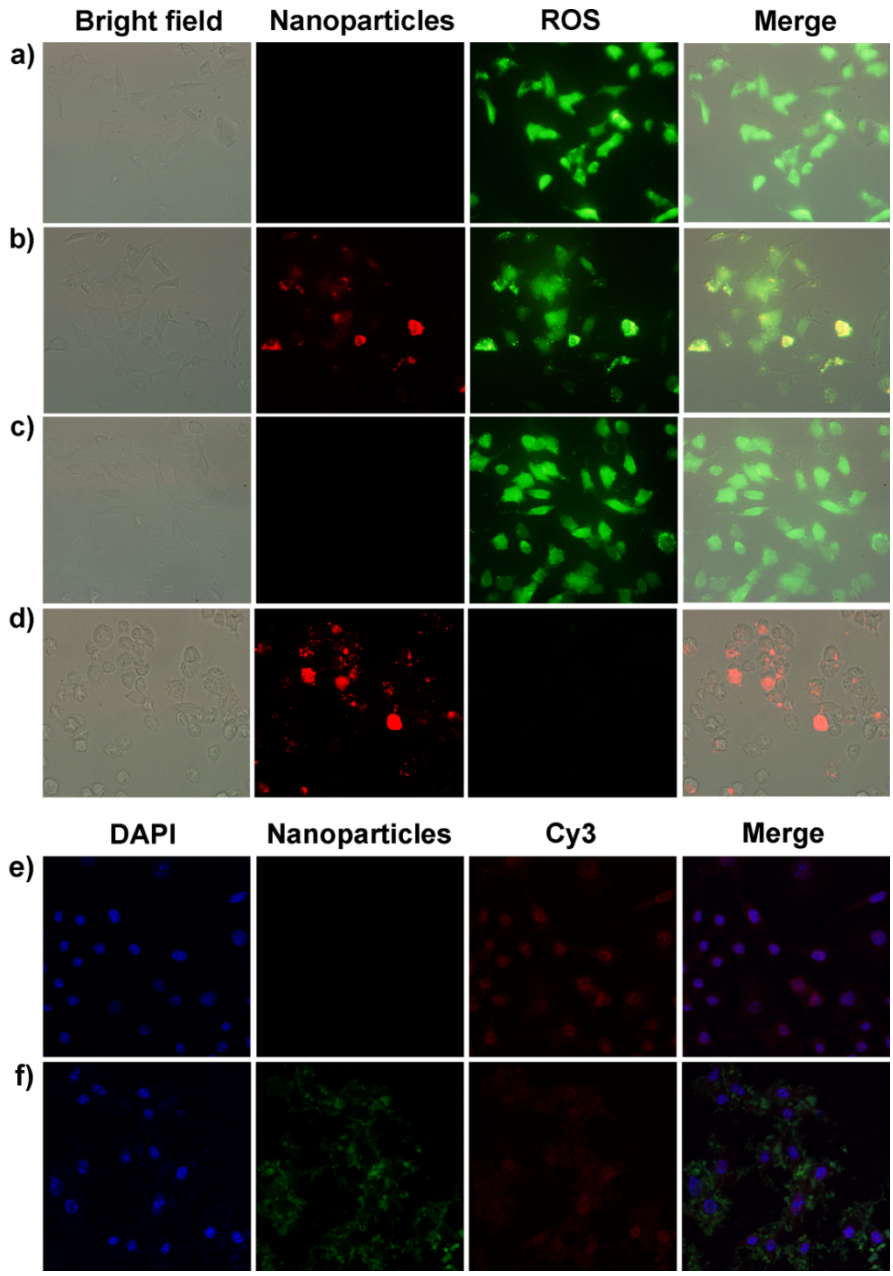

Fig. S9. Fluorescence microscope analysis of intracellular ROS generation in RAW264.7 cells (a-d). Cells were treated with positive control (Roseup, a); MSN@RITC-SP-LPS (b); SP-LPS (c); MSN@RITC (d). Analysis of nuclear translocation of NF-κB by Immunofluorescence staining (e-f). Cells were treated with SP-LPS (e) and MSN@FITC-SP-LPS (f). p65 expression was stained by the p65 antibody and a Cy3-conjugated secondary antibody. Nuclei were stained with DAPI and cells were imaged under a confocal laser scanning microscopy.

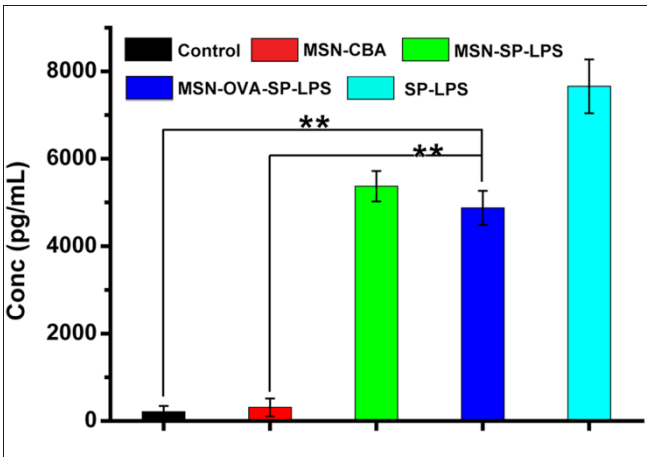

Fig. S10. The levels of TNF-α elicited by various stimulus and measured by ELISA method. **p＜0.005.

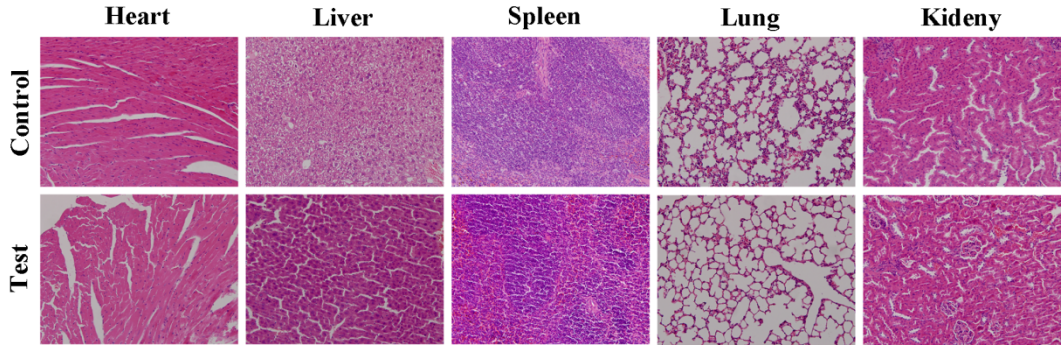

Fig. S11. H&E-stained tissue sections from major organs after intravenous injection of 0.9 wt% NaCl solution and MSN-SP-LPS.