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Supporting Information for

Enhanced antibacterial function of supramolecular artificial receptor-modified macrophage (SAR-Macrophage)

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Experimental Procedures

Materials

Allyl-α-D-mannopyranoside was purchase from Shanghai Qian Yan Biotech company, LIVE/DEAD™ BacLight™ Bacterial Viability Kit, for microscopy & quantitative assays was purchase from Thermo Fisher. 1-adamantanethiol, fluorescein isothiocyanate (FITC), Cy5 NHS ester, DCFHDA, diphenyltetrazolium bromide (MTT), lysogeny broth (LB) and other chemicals were all purchased from aladdin and used as received without further purification. Escherichia coli (*E. coli*, ATCC 25922) and Raw 264.7 mouse macrophage cells were purchased from American Type Culture Collection (ATCC, Manassas, VA).

The NMR spectra were acquired on a Bruker Ultra Shield 600 PLUS NMR spectrometer. Fluorescence spectra were obtained on a fluorescence spectrometer (Thermo Scientific Lumina). Fluorescence images were acquired by confocal laser scanning microscopy (CLSM, Leica TCS SP8, German) and inverted fluorescence microscopy (Olympus IX73). MTT assays were measured using a microplate reader (Infinite F200 Pro, TECAN). ROS detection assay and macrophage polarization experiments were both quantified by a flow cytometer (Beckman coulter).

Synthesis of mannose-ADA

The Allyl-α-D-mannopyranoside was conjugated with 1-adamantanethiol via a "click" reaction between Allyl-α-D-mannopyranoside (50mg, 0.23mmol.) and 1-adamantanethiol (50mg, 0.27mmol.) in quartz reaction tube containing 2 mL MeOH under 12 h UV irradiation. The excessive 1-adamantanethiol was removed by filtration. The filtrate was freeze dried to yeild mannose-ADA (75mg, 75% yield). ^{1}H NMR (600MHz, D2O) 1.72 (m, 3H), 1.87 (m, 6H), 2.09 (m, 6H), 2.83 (t, 2H), 3.66 (t, 2H), 3.56-3.98 (m, 6H), 4.88 (d, 1H) ppm. ^{13}C NMR (125MHz, D2O), δ 99.87, 72.85, 70.54, 70.00, 66.71, 60.89 56.73, 38.68, 36.18, 35.48, 28.35, 27.90.

Cell Culture

Raw 264.7 cells were provided by American Type Culture Collection (ATCC). The cells were cultured in DMEM medium containing 100 μg mL-1 streptomycin, 10% heatinactivated FBS, 100 U mL^{-1} penicillin, and maintained in a humidified incubator with 5% CO_2 at 37 °C.

Bacteria culture

E. coli was provided by American Type Culture Collection (ATCC). A single colony on LB agar plate for *E. coli* was transferred to 2 mL of liquid culture medium and grown at 37 °C for 10 h. The concentrations of bacteria were determined by measuring the optical density at 600 nm (OD 600) and 109 colony forming unit (CFU) of bacteria was transferred to 1.5 mL EP tube. Bacteria were harvested and washed by centrifugation at 7000 rpm for 3 min, followed by the removal of supernatant.

Anchoring *E. coli* with mannose-ADA

10 μL harvested *E. coli* (10^{9}CFU/mL) dispersing in 1 mL PBS were added 50 μg/mL mannose-ADA for shaking 30 mins, followed by centrifugation at 8000 rpm for 5 min. Then *E. coli* was collected and resuspended in PBS. Then cucurbit[7]uril (CB[7])-Cy5 was used to bind mannose-ADA modified *E. coli*. The fluorescence image was obtained using laser scanning confocal microscope.

Anchoring macrophage with DSPE-PEG-CB[7]

DSPE-PEG-CB[7] (DSPE-PEG-CB[7] (1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethylene glycol)-CB[7]) was synthesized according to our previous procedure.[1] Macrophages were incubated in cell culture and treated with 10 μM DSPE-PEG-CB[7] for 2 hours at 37℃, and SAR-Macrophage was obtained. After preparation CB[7]-Macrophage and washing away excess DSPE-PEG-CB[7], the supramolecular cells were incubated for another 1 h, 2 h, 4 h, 12 h and 24 h, respectively. Subcutaneously, ada-FITC was added and the cells were incubated for 2 min at room temperature. After following washes to remove unbound ada-FITC, fluorescence imaging experiments were performed on confocal laser scanning microscope (CLSM) to see the stability of DESP-PEG-CB[7].

Cytotoxicity studies

MTT assays were used to assess the cell viability of Raw 264.7 cells after incubation with various conc. of DSPE-PEG-CB[7]. 24 h later, 10 μL of MTT solution (5 mg/mL in PBS) was added into each well. After 4 h of incubation, 100 μL of DMSO was added to each well. The UV absorption was recorded using a plate reader.

A volume of 50 μL of harvested bacteria was added into a 96-well plate and mixed with 50 μL of LB containing different conc. of mannose-ADA, giving a final bacterial concentration of 5×10^{5}CFU/mL for incubation for 24 h. Testing concentration varied in half fold per a standard protocol. A growth control group with only bacterial solution and a sterile control group with only growth medium were carried out at the same time.

Recognition of *E. coli* by macrophages

10 μL harvested GFP-expressing *E. coli* (10^9 CFU/mL) dispersing in 1 mL PBS were added to the Raw 264.7 cell culture in confocal dish (MOI=10/MOI=1), followed by different incubation time in 5% CO_2 humidity at 37 °C. After that, the cell culture medium was washed with PBS twice followed by CLSM.

Intracellular ROS Detection.

The ROS generation inside the cells upon recognition of *E. coli* was studied using a cell permeable indicator DCFHDA. Raw 264.7 cells were seeded in 12-well and then infected with different treatment of *E. coli* for 4 h followed by washing with PBS two times, then DCFHDA (10 μmol) was loaded into the cells. After 30 min incubation, the cells were washed with PBS and studied by CLSM and quantified by flow cytometry.

Intracellular bacterial killing study

The live/dead staining assay of isolated intracellular *E. coli*. After incubation of RAW 264.7 with *E. coli* in various matching formulations (CB[7](-),ADA(-)), (CB[7](+),ADA(-)), (CB[7](-),ADA(+)) and (CB[7](+),ADA(+)), respectively, for 4 h; the extracellular bacteria were washed off and the infected macrophages were incubated for additional 18 h, before the intracellular *E. coli* were isolated from macrophage. The macrophages with engulfed bacteria were lysed with PBS containing 1% Triton-X. The intracellular bacterial were stained by LIVE/DEAD™ BacLight™ Bacterial Viability Kit for CLSM.

Macrophage polarization

Raw 264.7 cells were seeded in 12-well and then infected with different treatment of *E. coli* for 4 h. After washing with PBS, the cells were stained with the following antibodies: FITC labeled CD11c antibody and APC labeled CD206 antibody. Then, macrophages were collected and analyzed by flow cytometry to detect the ratio between M1 macrophage and M2 macrophage. The uninfected macrophages were served as control group.

Antibacterial experiments

The bacteria were diluted with broth to 10^5 CFU/mL. The antibacterial abilities of different treatment against bacteria were determined by using optical density at 600 nm (OD600). *E. coli* or UPEC were incubated with different treatments with amount of 10^5/mL cells that were dispersed in PBS at 37 °C for 4 h. The absorbance at 600 nm was recorded, and the bacteria without treament were used as control. The culture medium without bacteria was considered as the background.

In vivo Zebrafish study

All animals used in the procedures were handled in strict accordance with the Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the Animal Ethics Committee, the University of Macau.

Wild-type AB zebrafish were used for this experiment. To prepare for microinjection, glass micropipettes will be fabricated with a micropipette puller and were subsequently cut with a scalpel to construct an opening of approximately 30 μm, slightly larger than the size of a suspended cell. Zebrafish larvae was anesthetized with 0.003% tricaine methane sulfonate (MS-222) and positioned on a wet agarose microinjection pad. For macrophage, approximately 100-200 cells (5 nL, 1×10^6 cells/50 μL) with DiD fluorescent labeling were injected into the common cardinal vein (CCV) of the fish larvae (48 hpf). For bacteria, approximately 1000-2000 cells (3 nL, 2×10^7 cells/50 μL) were injected into the vein for circulation. Then, embryos were observed by fluorescent microscopy to monitor the binding between macrophages and bacteria in vivo. The quantitative results were analysed by imageJ.

In vivo infectious mice study

Balb/c mice (8-week old) were used in this study. All animals used in the procedures were handled in strict accordance with the Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the Animal Ethics Committee, the University of Macau. The mice were randomly divided into five groups (n = 3 per group): PBS, (CB[7](-), ADA(-)), (CB[7](+), ADA(-)), (CB[7](-), ADA(+)) and (CB[7](+), ADA(+)). After anaesthesia and removing the dorsal hair, six 6-mm-diameter full-thickness wounds were generated on the back of mice using a biopsy punch. The wounds were infected by 10 μL *E. coli* suspension (1×10^9 CFU/mL) per wound and covered with Tegaderm (3M) for 24 h. After mice underwent 4 h infection, mannose-ADA (10 μL, 50 μg/mL) and SAR-Macrophage (100 μL, 10^6/mL) were sequentially injected locally with 1 h gap for the (CB[7](+), ADA(+)) group, and other groups of mice were treated in a similar manner. The wounds were observed daily and photos were taken and the area of wounds were measured on Day 0, 1, 3, 5, 7, 9 and 11. For one batch of the mice, on Day 5, the wounds and surrounding skin of mice were harvested and fixed with 4% paraformaldehyde overnight. After homogenization, samples were plated into LB medium to determine CFU. The wound and skin were then embedded in paraffin, sectioned, and stained with haematoxylin & eosin for histological examinations.

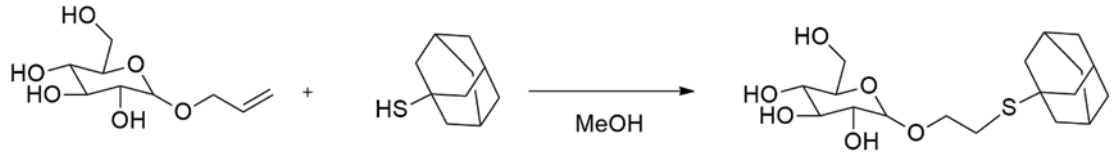

Figure S1. Synthetic route toward mannose-ADA.

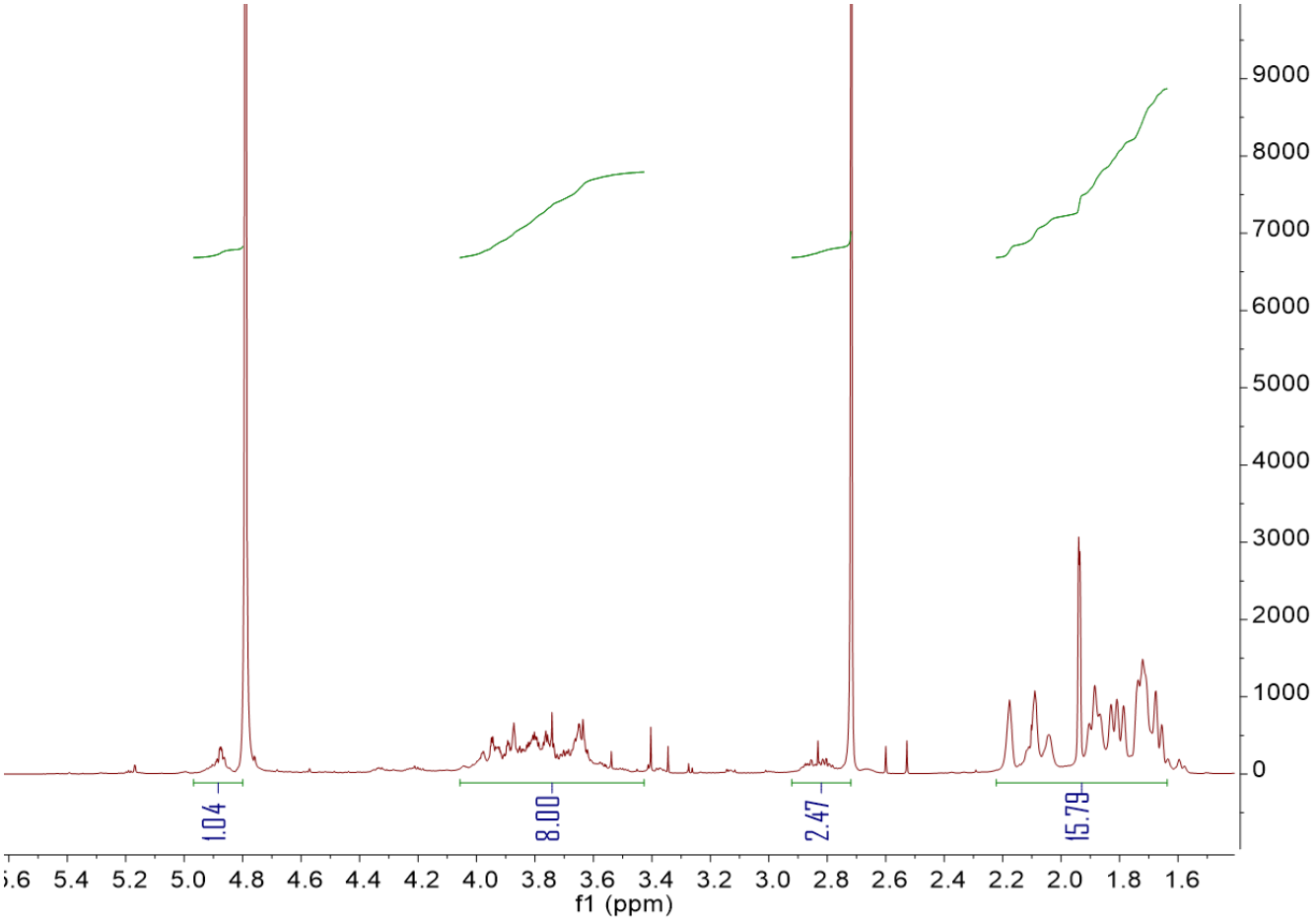

Figure S2. ^{1}H NMR spectrum of mannose-ADA in D_2O.

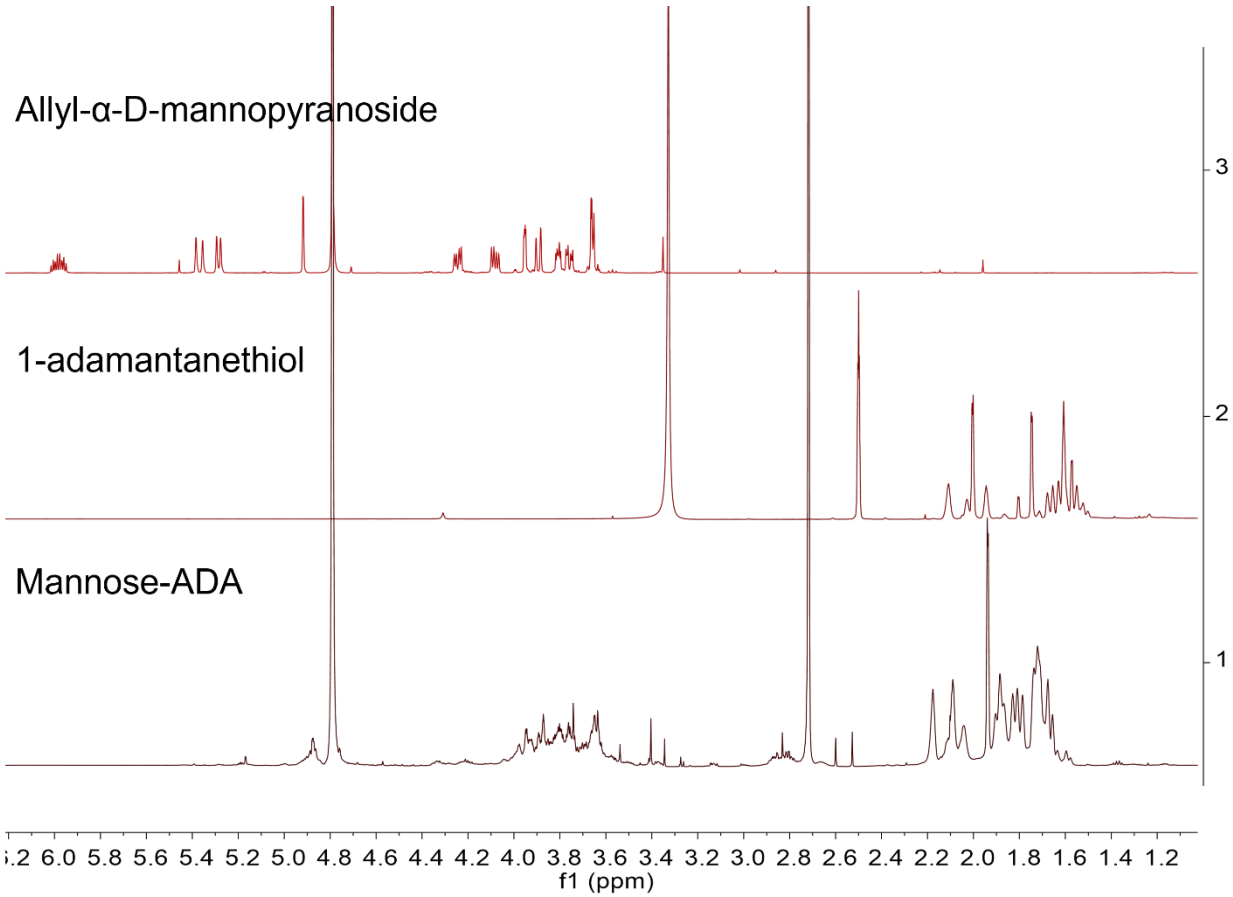

Figure S3. ^{1}H NMR spectra of allyl-α-D-mannopyranoside, 1-adamantanethiol and mannose-ADA in D_2O.

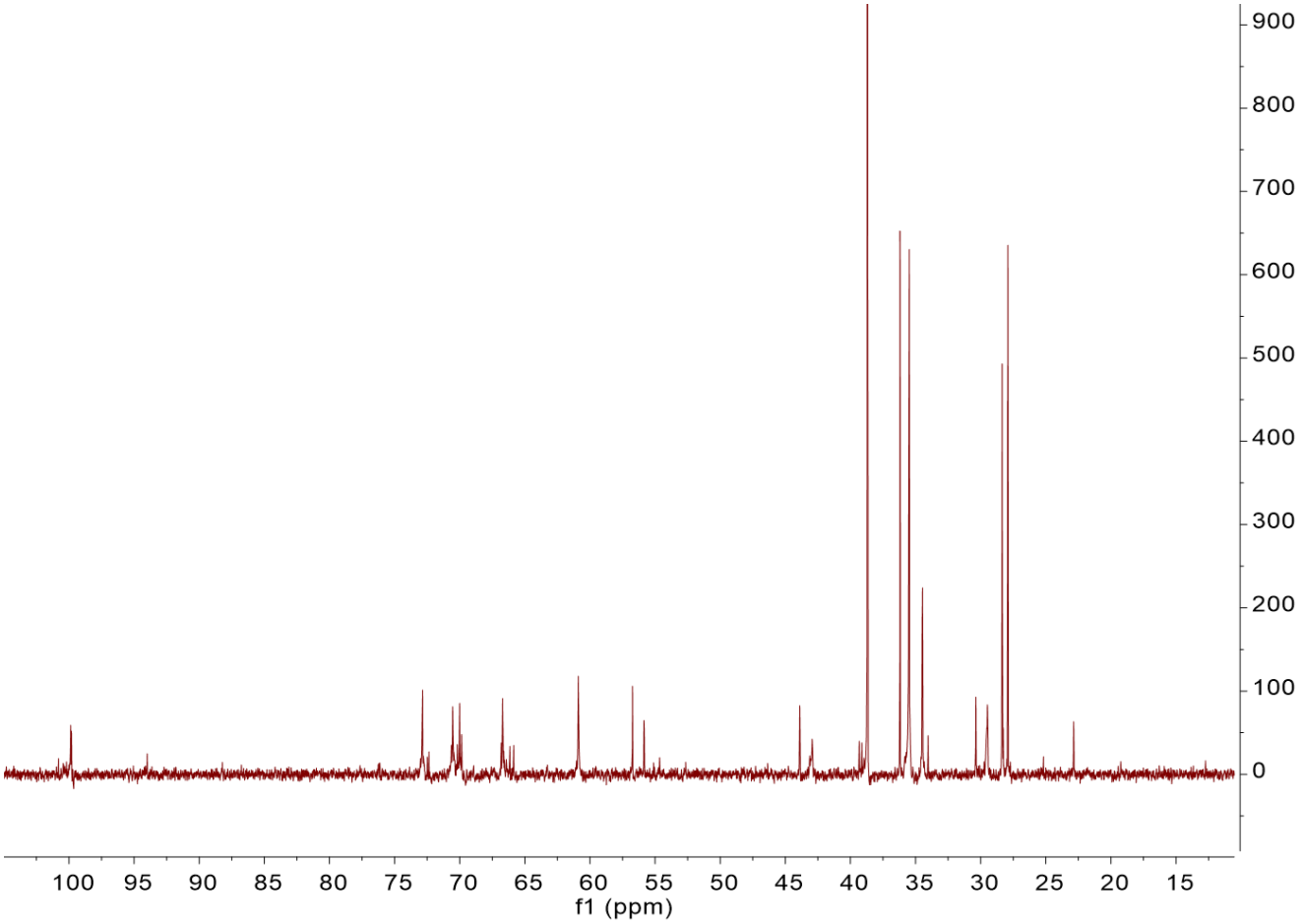

Figure S4. ^{13}C NMR spectrum of mannose-ADA in D_2O.

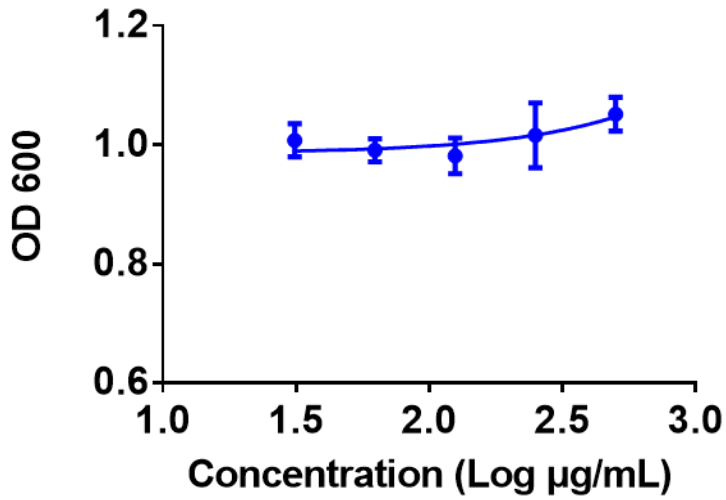

Figure S5. Cell viability of mannose-ADA upon *E. coli* after incubation for 24 h.

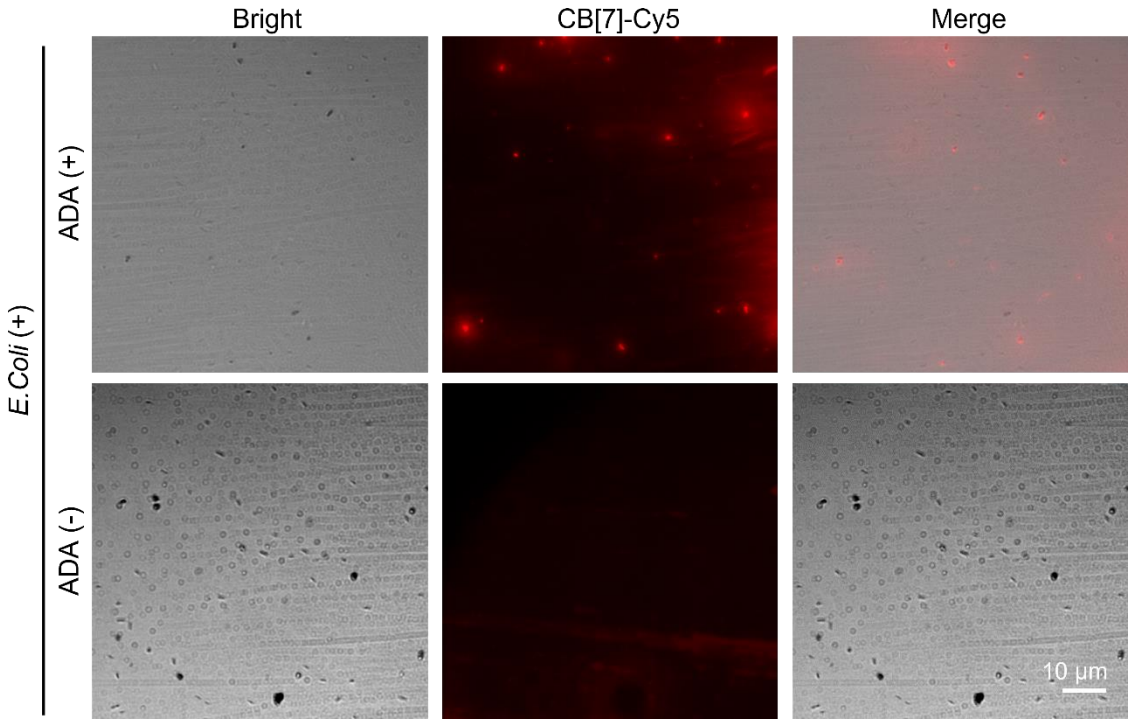

Figure S6. Fluorescence image of *E. coli* with or without incubation with mannose-ADA and subsequently treated with CB[7]-Cy5 for 5 min.

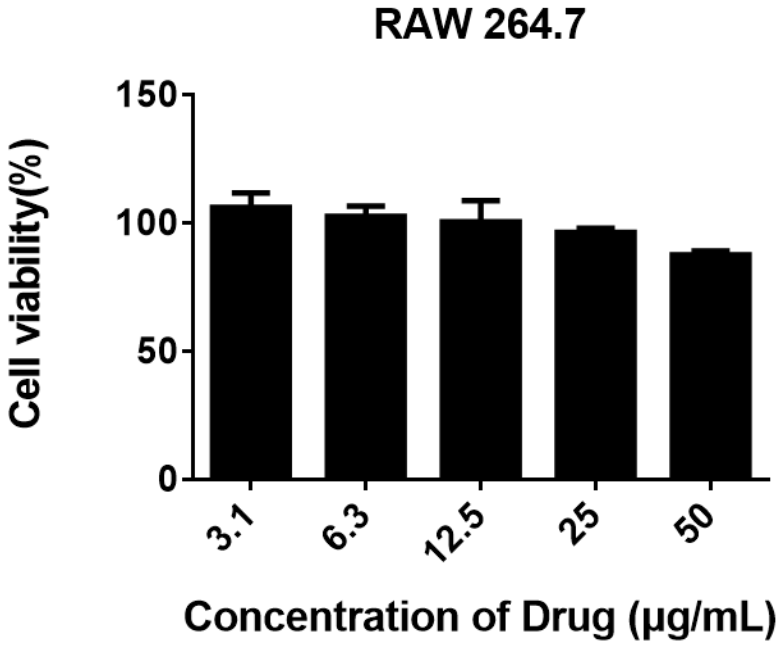

Figure S7. Cell viability of RAW 264.7 after incubation with DSPE-PEG-CB[7] for 24 h.

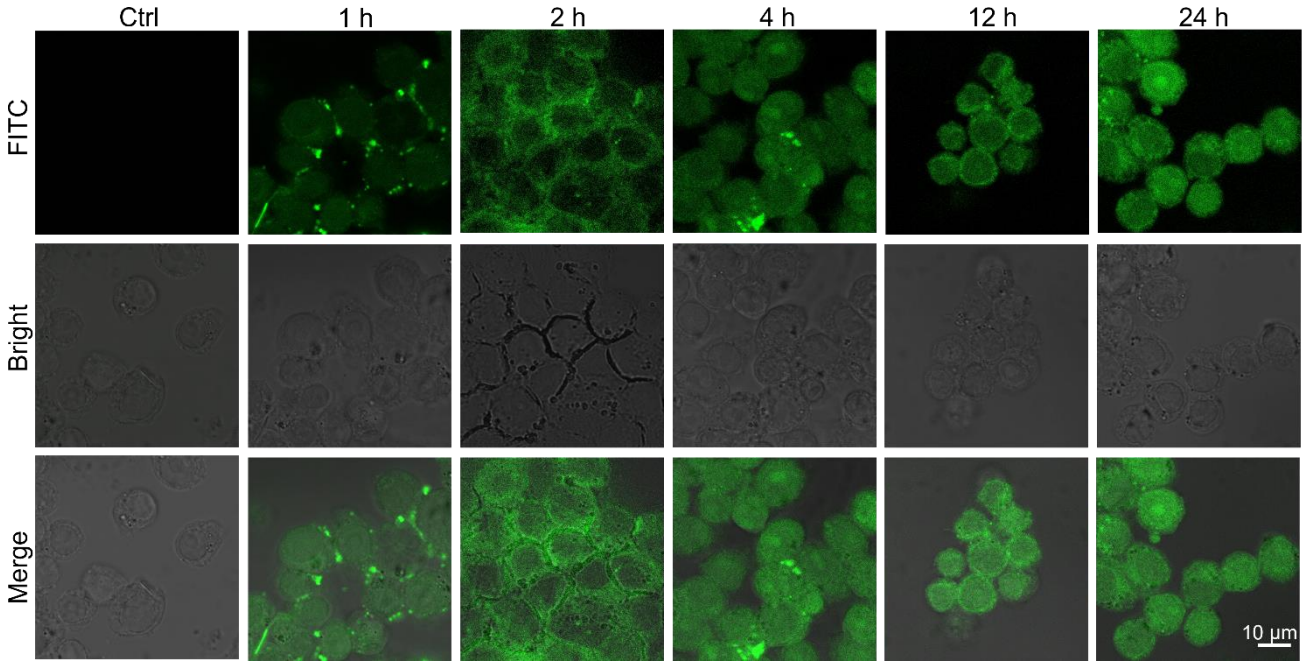

Figure S8. The fluorescence images of RAW264.7 cells incubated with DSPE-PEG-CB[7] for 1, 2, 4, 12 and 24 h, respectively, then treated with ada-FITC for 5 mins.

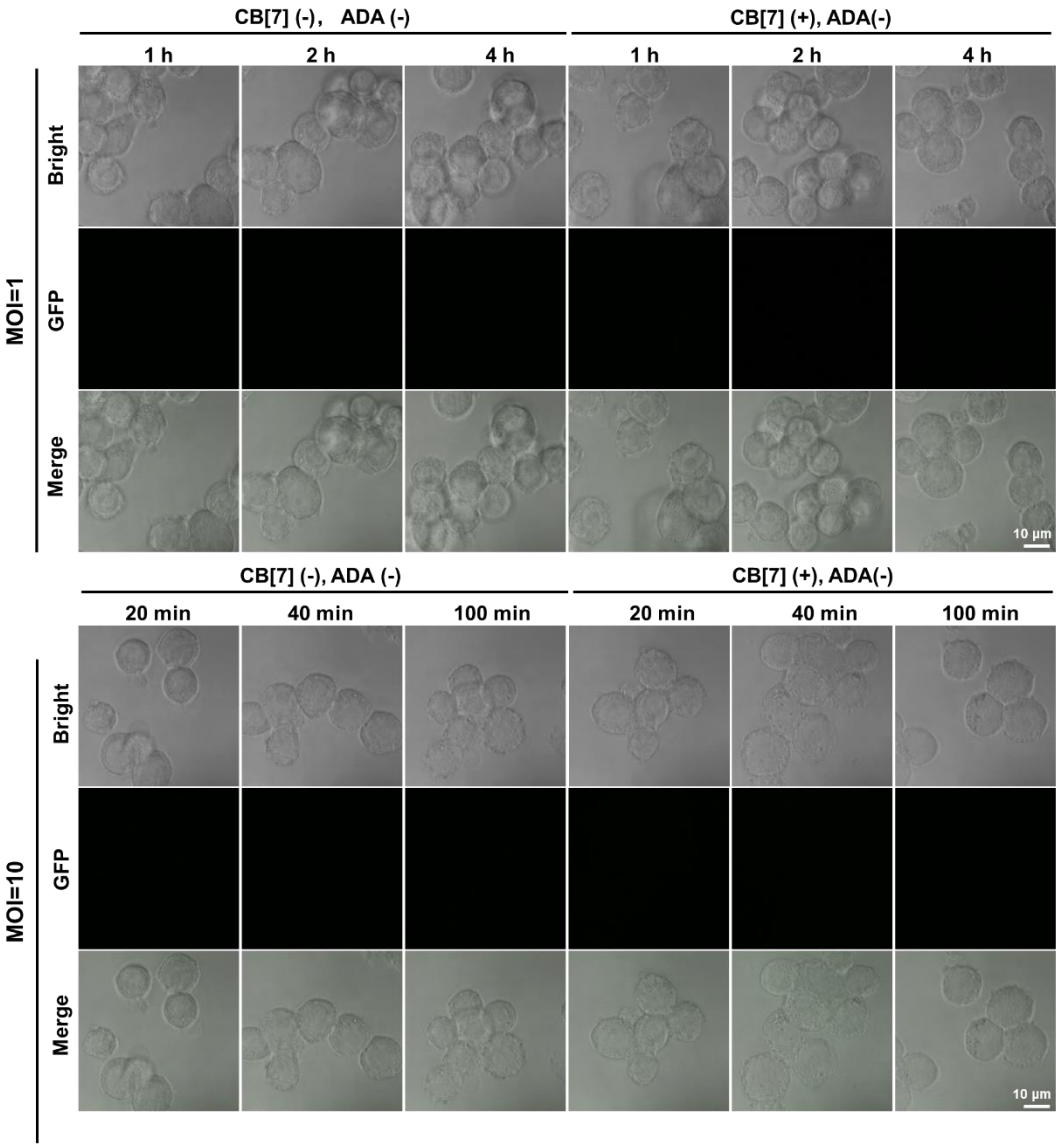

Figure S9. A) CLSM images of macrophage infected with *E. coli* (MOI = 1) in the (CB[7] (-), ADA (-)) and (CB [7] (+), ADA (-)) groups for 1, 2 and 4 h, respectively. B) CLSM images of macrophage infected with *E. coli* (MOI = 10) in the (CB[7] (-), ADA (-)) and (CB[7] (+), ADA (-)) group for 20, 40 and 100 min, respectively.

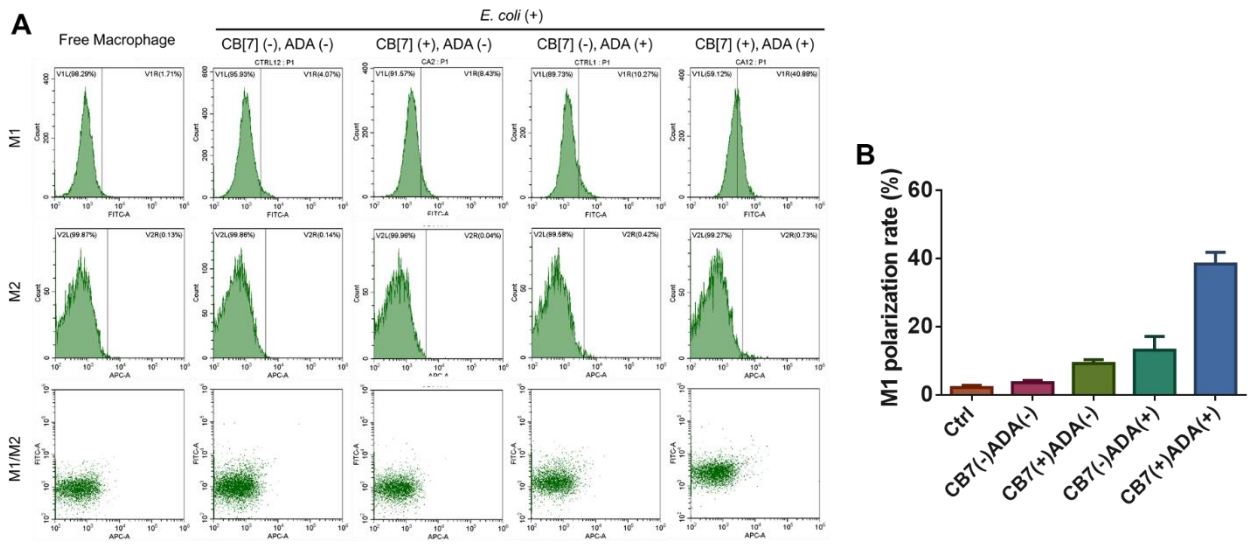

Figure S10. A) Flow cytometry analysis of polarization of macrophage after incubation with DMEM, (CB[7](-),ADA(-)), (CB[7](+),ADA(-)), (CB[7](-),ADA(+)) and (CB[7](+),ADA(+)), respectively. B) Quantitative result from A.

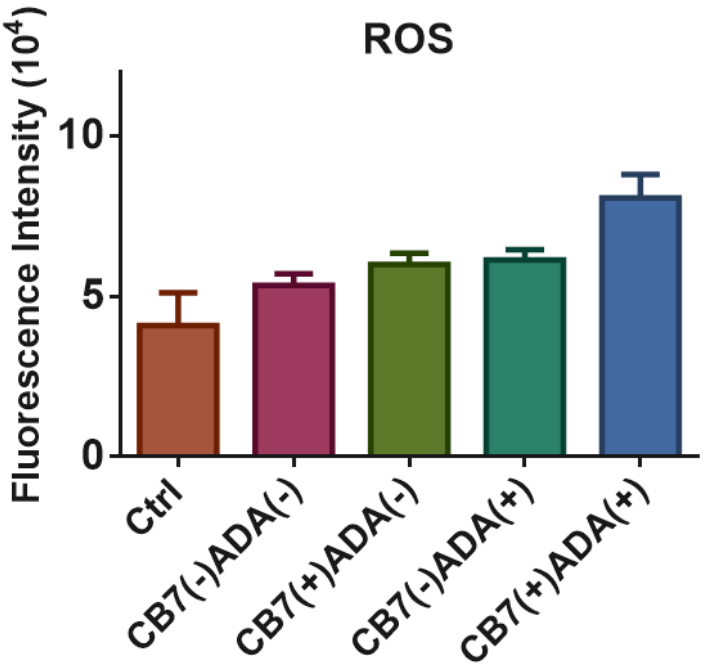

Figure S11. Quantitative flow cytometry analysis of generation of intracellular ROS after incubation with DMEM, (CB[7](-),ADA(-)), (CB[7](+),ADA(-)), (CB[7](-),ADA(+)) and (CB[7](+),ADA(+)), respectively.

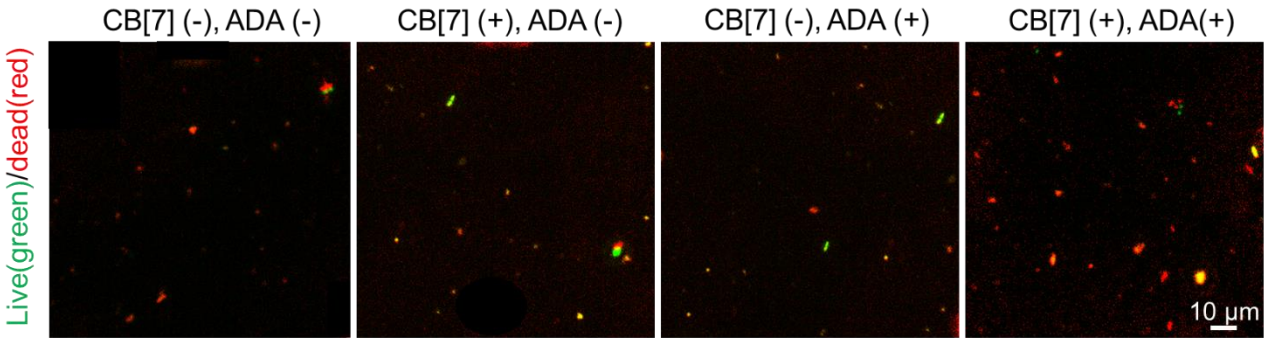

Figure S12. The live/dead staining assay of isolated intracellular *E. coli*. After incubation of RAW 264.7 with *E. coli* in various matching formulations (CB[7](-),ADA(-)), (CB[7](+),ADA(-)), (CB[7](-),ADA(+)) and (CB[7](+),ADA(+)), respectively, for 4 h; the extracellular bacteria were washed off and the infected macrophages were incubated for additional 18 h, before the intracellular *E. coli* were isolated from macrophage.

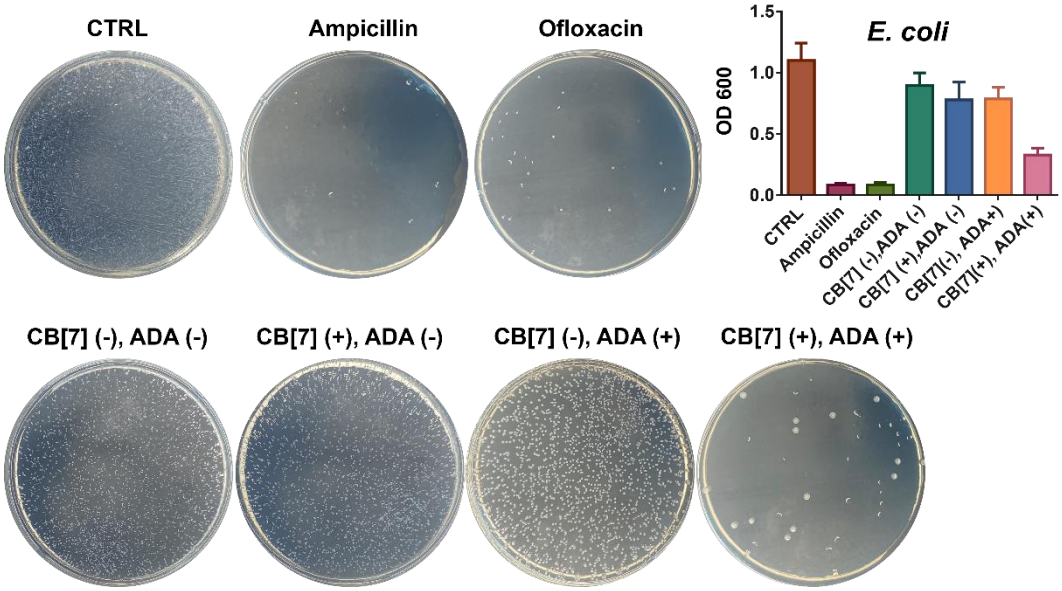

Figure S13. Photo and quantitative analysis of bacterial colony of *E. coli* incubated with PBS, ampicillin, ofloxacin, and macrophage (CB[7](-), ADA(-)), (CB[7](+), ADA(-)), (CB[7](-), ADA(+)) and (CB[7](+), ADA(+)), respectively (the concentration of antibiotics was 50 μg/mL, and the amount of macrophage was 10^5/mL).

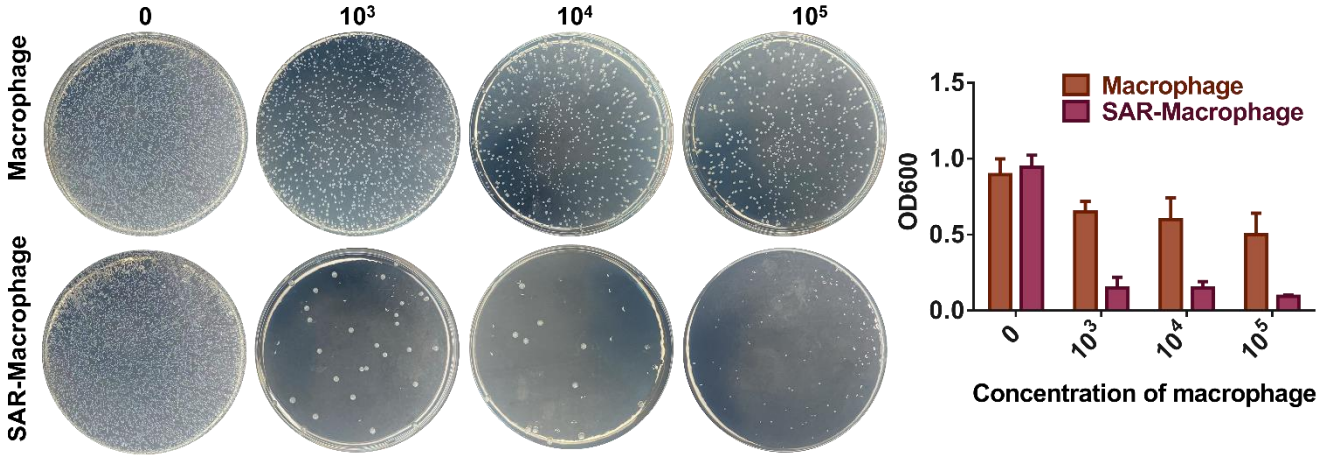

Figure S14. Photo and quantitative analysis of bacterial colony of *E. coli* incubated with different quantity of regular macrophage and SAR-Macrophage, respectively (the concentration of *E. coli* was fixed at 10^5 CFU/mL).

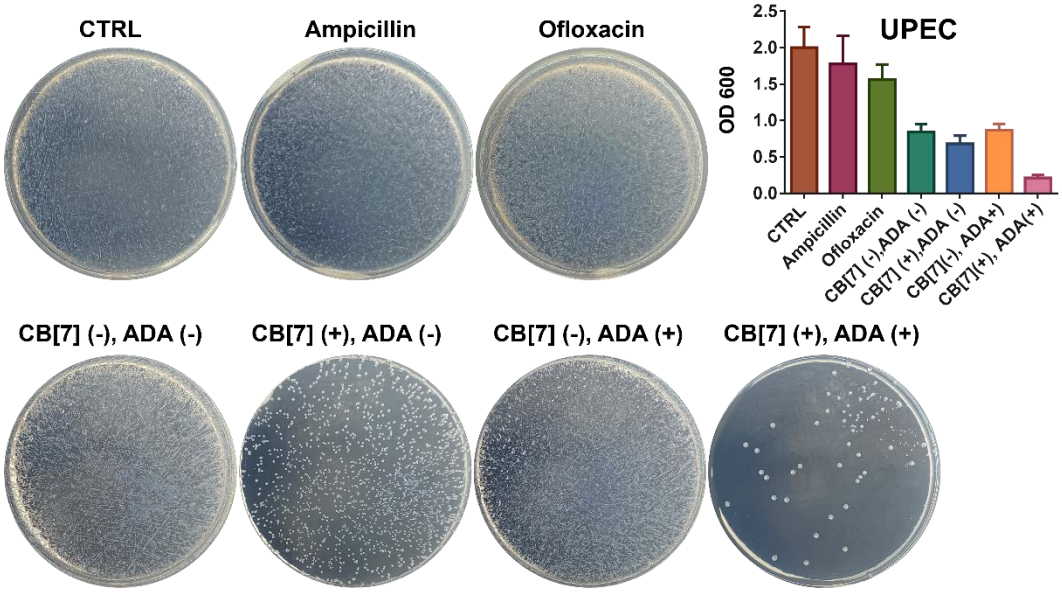

Figure S15. Plate counting photos and quantitative analysis of bacterial colony of UPEC incubated with PBS, ampicillin, ofloxacin, and macrophage (CB[7](-), ADA(-)), (CB[7](+), ADA(-)), (CB[7](-), ADA(+)) and (CB[7](+), ADA(+)), respectively (the concentration of antibiotics was 50 μg/mL, and the amount of macrophage was 10^5/mL).

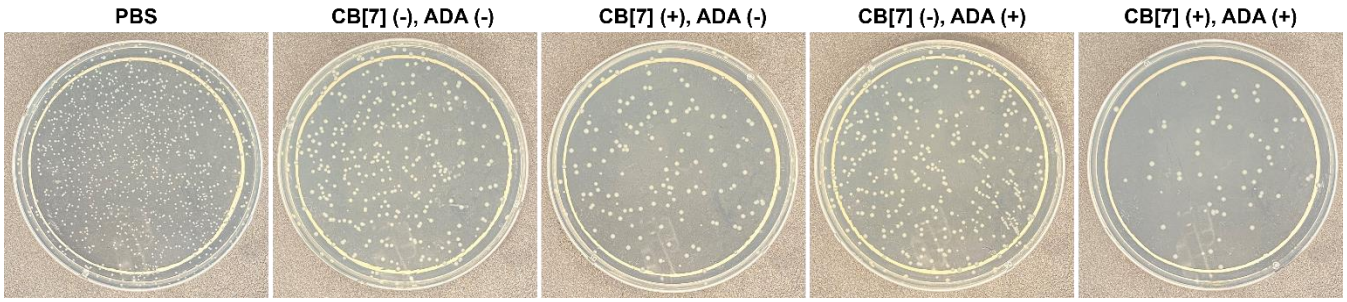

Figure S16. Photographs of the bacterial colony of *E. coli* isolated from the wound tissue after treatment for 5 days in each group.

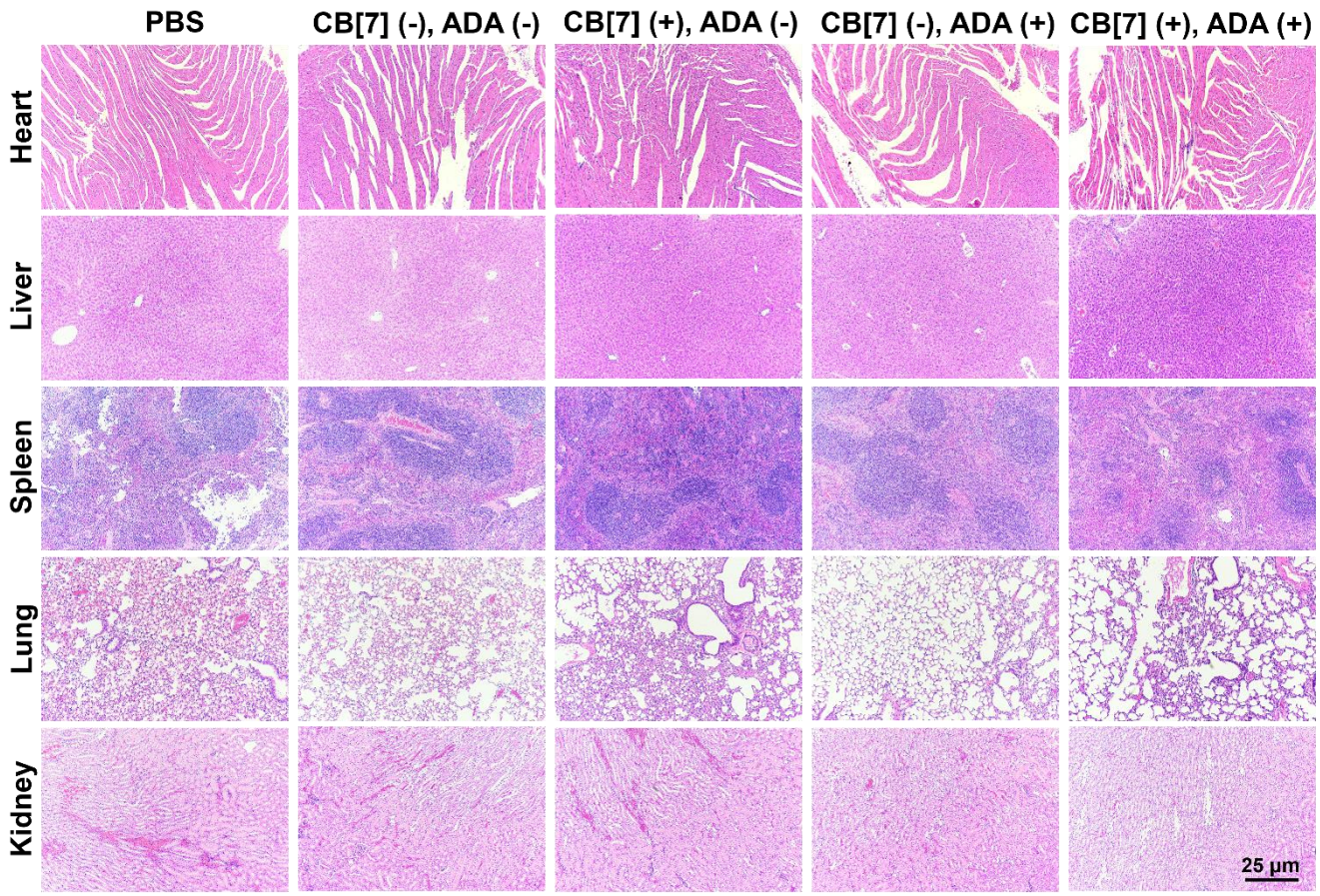

Figure S17. Biosafety evaluation of various matching formulations (CB[7](-),ADA(-)), (CB[7](+),ADA(-)), (CB[7](-), ADA(+)) and (CB[7](+),ADA(+)), respectively in major organs (heart, liver, spleen, lung, and kidney) after 5 days treatment.

Reference

1. Gao, C.; Cheng, Q.; Li, J.; Chen, J.; Wang, Q.; Wei, J.; Huang, Q.; Lee, S. M. Y.; Gu, D.; Wang, R., Supramolecular Macrophage-Liposome Marriage for Cell-Hitchhiking Delivery and Immunotherapy of Acute Pneumonia and Melanoma. *Adv. Funct. Mater.* **2021**, 2102440.