Supporting Information

Improving STING Agonist Delivery for Cancer Immunotherapy Using Biodegradable Mesoporous Silica Nanoparticles

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Supporting methods and materials

Materials: CDA was purchased from InvivoGen (Cat.no.: tlrl-nacda). THP1-Blue ISG cells were purchased from InvivoGen. Antibodies used for staining of cells were BV605-anti-mouse CD45 (103155; BioLegend), BV605-anti-mouse CD8α (563152; BD), APC-anti-mouse CD3ε (100312; BioLegend), FITC-anti-mouse CD3 (100204; BioLegend), PECY7-anti-mouse NKG2D (25-5882-82; eBioscience), APC-anti-mouse CD107α (560646; BD Biosciences), PE-anti-mouse NK1.1 (12-5941-82; eBioscience), PE-anti-mouse CD11c (50-102-20, eBioscience), FITC-anti-mouse CD80 (561954; BD Biosciences), APC-anti-mouse CD40 (50-149-60; eBioscience), and PECY7-anti-mouse CD86 (560582; BD Biosciences).

Activation of bone marrow-derived dendritic cells (BMDCs) and melanoma cells: Mouse BMDC were harvested from femurs of C57BL/6 mice. BMDC precursor cells were cultured with GM-CSF in 37 °C

with 5% CO_2. The cells were used between 7-11 days of culture. Mature BMDCs were collected and seeded with $5x10^4$ cells per well ($1x10^5$ cells per well for C-178 mediated STING inhibition study) of 96-well tissue culture plates, followed by overnight incubation. Mouse melanoma cells (B16F10 and B16F10OVA) were seeded ($5x10^4$ cells/well) in 96-well tissue culture plates and were incubated overnight. After removing the culture media and two times of washing with PBS, fresh culture media containing 10 μg/ml of CDA were added. After 6 hours of incubation, supernatants were collected and stored at -80 °C until analyses for cytokines and chemokines detection by ELISA.

STING activation of THP1-Blue ISG: THP1-Blue ISG cells were seeded on a 96-well tissue culture plate with 10^5 cells per well. CDA was added at indicated concentrations for overnight incubation. Culture media were collected for measurement of secreted embryonic alkaline phosphatase (SEAP), a reporter protein secreted in response to IFN regulatory factor (IRF) activation, using QUANTI-Blue Solution (SEAP detecting reagent; InvivoGen). After 30 min incubation, the solution was measured at 620 nm for quantification of STING activation.

Blood sample processing: Gel-filled blood collection tubes (NC9991903; Sarstedt Inc.) were used to obtain sera. For analyses using flow cytometry, blood samples were collected in an EDTA-coated vacutainer (02-669-33; BD). ACK lysis buffer was used to remove red blood cells, followed by twice of washing with FACS buffer (1% BSA in PBS). CD16/32-blocking antibody (50-112-9520; eBioscience) and staining antibodies were sequentially added and stained with a viability dye (eFlour 450; eBioscience). Cells were fixed using 4% formaldehyde and washed with FACS buffer twice before running flow cytometric analysis (ZE5 Flow Cytometer; BioRad).

Tumor tissue processing for tumor microenvironment (TME) analyses: Tumor tissue samples were cut into small pieces (1-2 mm) using scissors. One mg/ml of Collagenase type IV (C5138; Sigma-Aldrich) and 100 U/ml of DNase I (D4263; MilliporeSigma) were added and incubated in 37 °C with shaking. After passing the cells through a 40 µm strainer, cells were centrifuged at 700 g for 5 min, followed by washing twice with PBS. After fixing cells with 4% formaldehyde and washing them twice FACS buffer, the samples were added with CD16/32-blocking antibody and stained with fluorophore-labeled antibodies for flow cytometric analysis. In a subset of studies, we also added cell-counting beads (C36950; Invitrogen) in order to count the number of immune cells within the TME.

Supporting figures

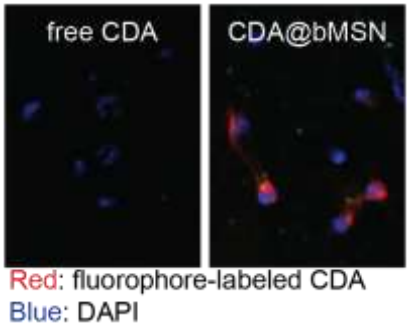

Figure S1. Enhanced uptake of CDA by B16F10 melanoma cells. B16F10 cells were treated with 5 µg/ml of fluorophore-labeled CDA either in a free form or in a loaded form in bMSN for 2 hours. Images were taken using a confocal microscope.

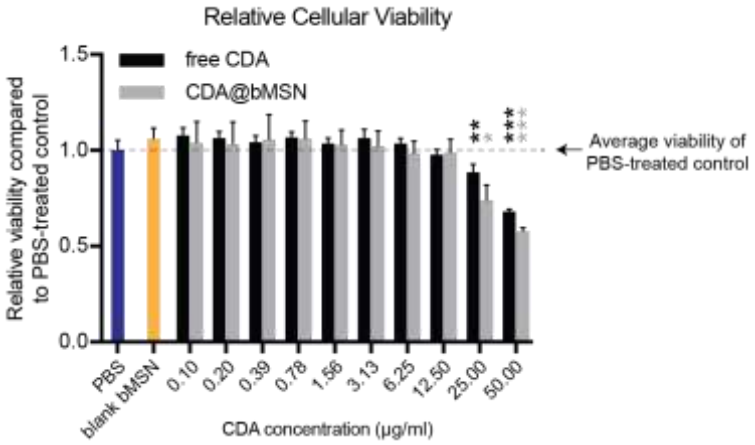

Figure S2. Cytotoxicity testing of CDA using a human monocyte-derived cell line, THP1-Blue ISG. THP1-Blue ISG cells were seeded in a 96-well tissue culture plate in a $5x10^4$ cells/well concentration. CDA in either free or bMSN-loaded form was treated in different CDA concentrations, then incubated overnight at 37 °C with 5% CO2. Cell viability was measured using a cell counting kit (96992; Sigma-Aldrich). Both forms of CDA did not show significant cytotoxicity to the cells until 12.5 µg/ml concentration.

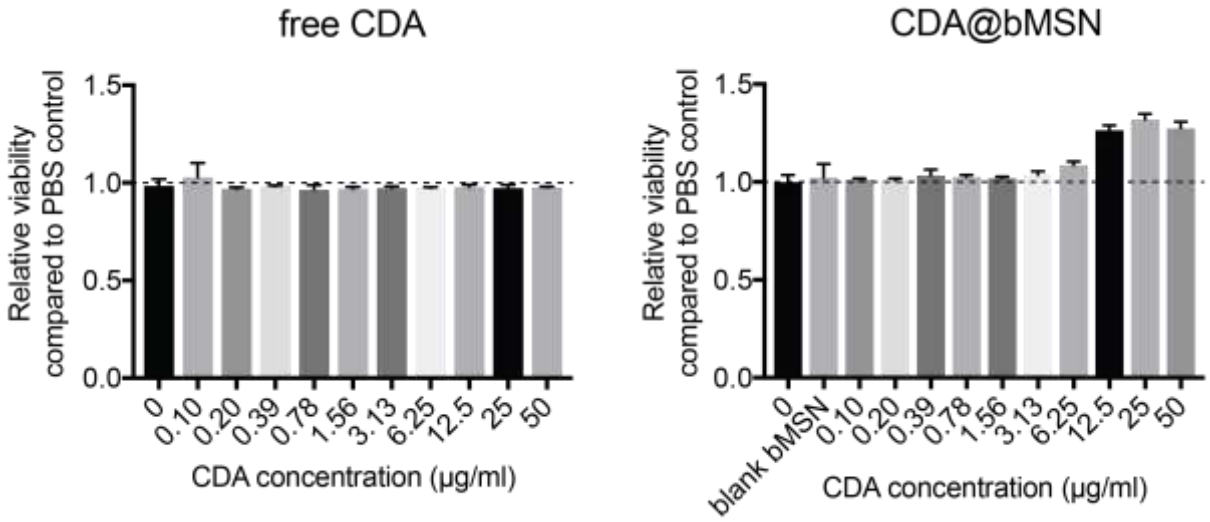

Figure S3. Cytotoxicity testing of CDA@bMSN on CD8+ T cells. CD8+ T cells were enriched from splenocytes collected from C57BL/6 mice. 100,000 cells were seeded per well of a 96-well plate and

treated with either free CDA or CDA@bMSN. After 4 hrs, CDA from the culture media was removed and, cell viability was measured.

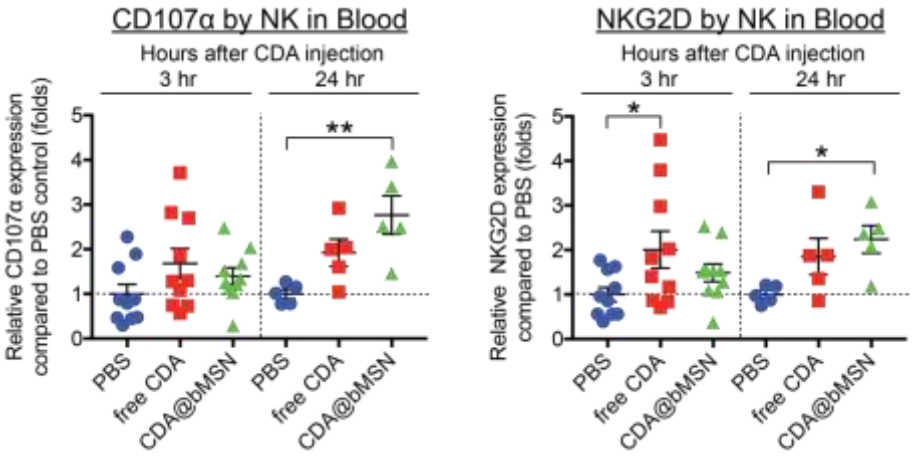

Figure S4. Activation of NK cells in blood circulation after intratumoral injection of 2 μg CDA in B16F10OVA-bearing mice. B16F10OVA-bearing mice were intratumorally injected with either free CDA or CDA@bMSN on day 6 after tumor inoculation. After 3 and 24 hours of the treatment, blood samples were collected for flow cytometric analysis.

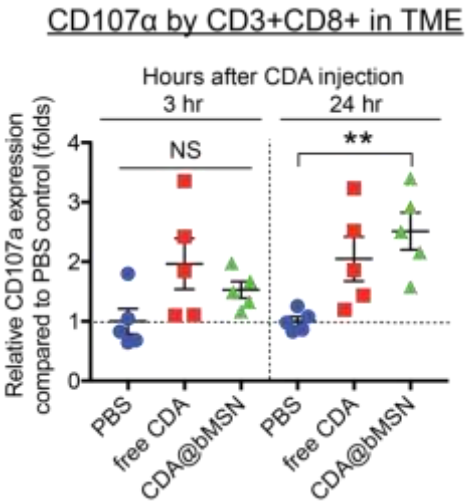

Figure S5. Intratumoral treatment of CDA@bMSN significantly elevates the expression level of CD107α on intratumoral CD8+ T cells, compared with free CDA.