Supporting Information

Single-component Lipid Nanoparticles for Engineering SOCS1 Gene-silenced Dendritic Cells to Boost Tumor Immunotherapy

Zexuan Yu[†] [a], Mengtong Wu[†] [a], Yingshuang Huang [a], Yishu Wang [a], Yijun Chen [a], Qiulin Long [a], Ziming Lin [a], Lingjing Xue [a], Caoyun Ju[*] [a], Can Zhang[*] [a, b]

[a] State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Advanced Pharmaceuticals and Biomaterials, China Pharmaceutical University, Nanjing 210009, P.R. China

[b] Chongqing Innovation Institute of China Pharmaceutical University, Chongqing 401135, China

[*] Correspondence to:

Can Zhang, Ph.D., State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Advanced Pharmaceuticals and Biomaterials, China Pharmaceutical University, Nanjing, P.R. China

E-mail: zhangcan@cpu.edu.cn

Caoyun Ju, Ph.D., State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Advanced Pharmaceuticals and Biomaterials, China Pharmaceutical University, Nanjing, P.R. China

E-mail: jucaoyun@cpu.edu.cn

[†] Authors contributed equally to this work

Supporting Figures

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Figure S18. Histological images of the H&E-stained organs collected from the mice treated with indicated formulations. Scale bar: 100 μm.

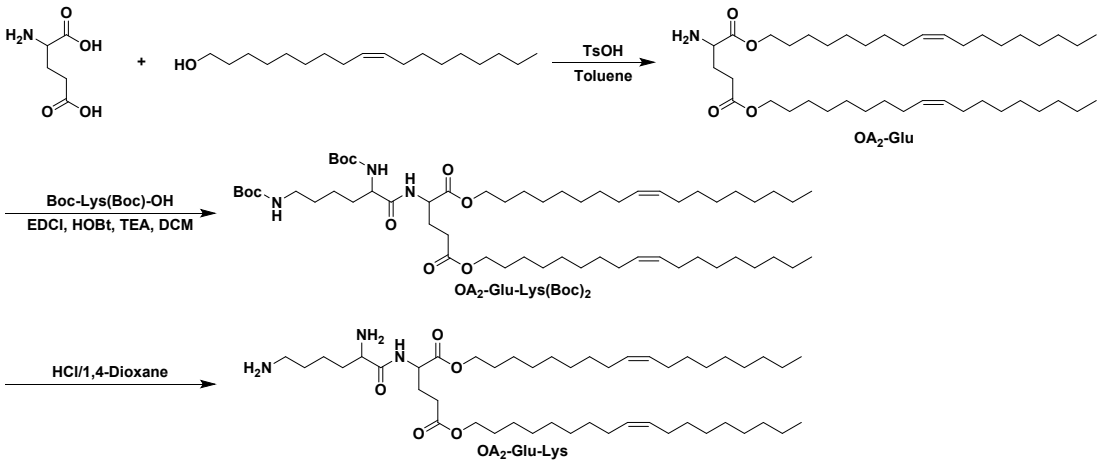

Figure S1. Scheme of OA2 lipid synthesis.

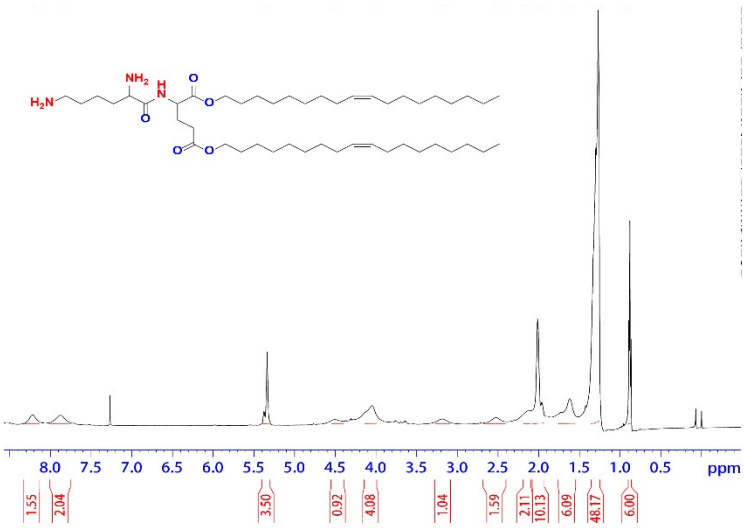

Figure S2. ^{1}H NMR spectra of OA2 lipid.

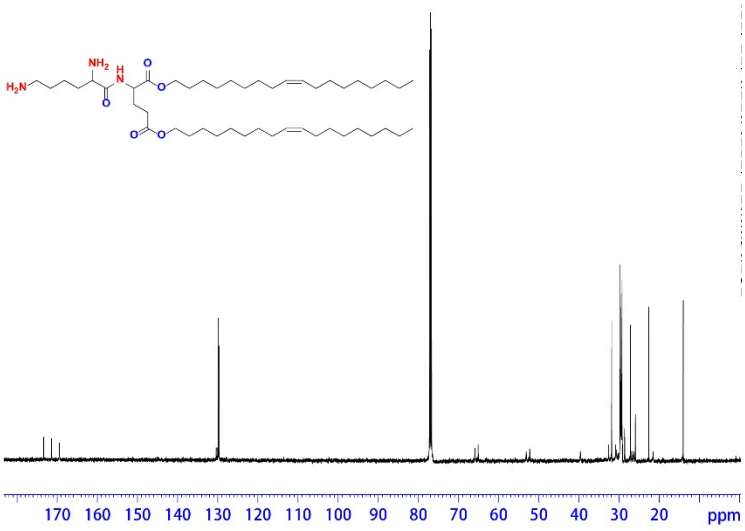

Figure S3. ^{13}C NMR spectra of OA2 lipid.

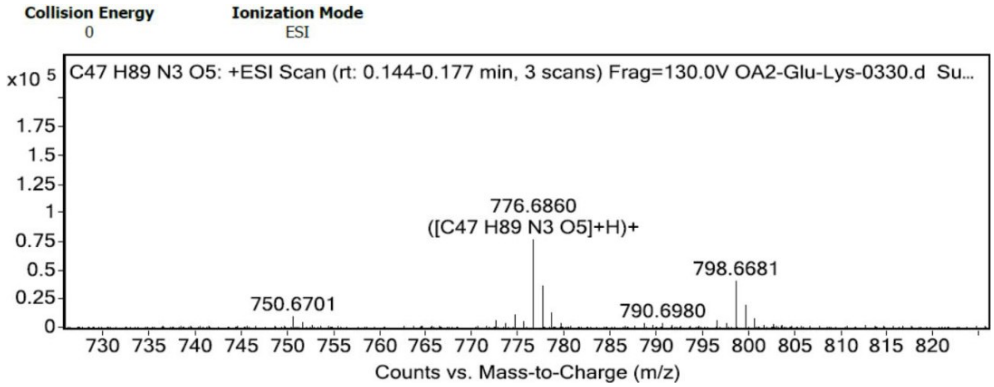

Figure S4. HRMS spectra of the OA2 lipid.

A

Characterization	OA2 LPs
Particle size (nm)	128.0 ± 0.1
PDI	0.14 ± 0.04
Zeta potential (mV)	43.2 ± 1.2

B

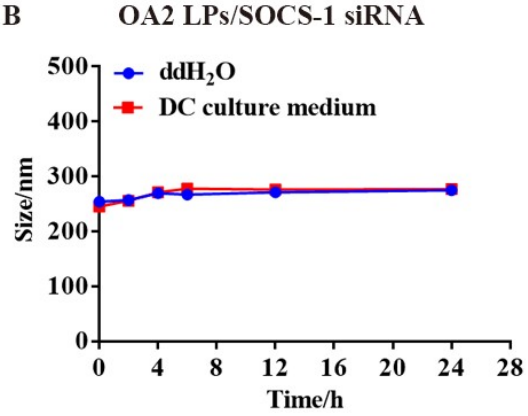

Figure S5. Characterization of OA2 LPs and *in vitro* stability of OA2 LPs/SOCS1-siRNA. (A) Mean particle sizes, PDI, and zeta potentials of OA2 LPs. (B) Change in the particle size of OA2 LPs/SOCS1-siRNA after incubation in the presence of ddH_2O or DC culture medium over time (Mean ± SD, n = 3).

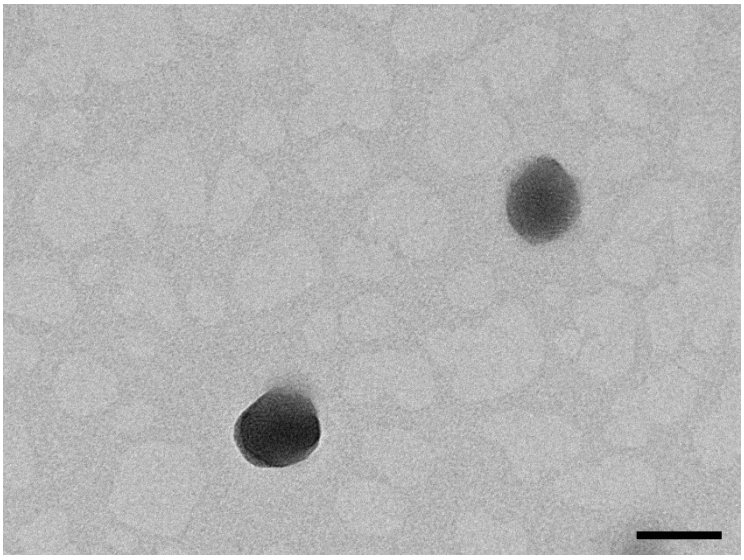

Figure S6. TEM image of OA2 LPs/SOCS1-siRNA. Bar: 100 nm.

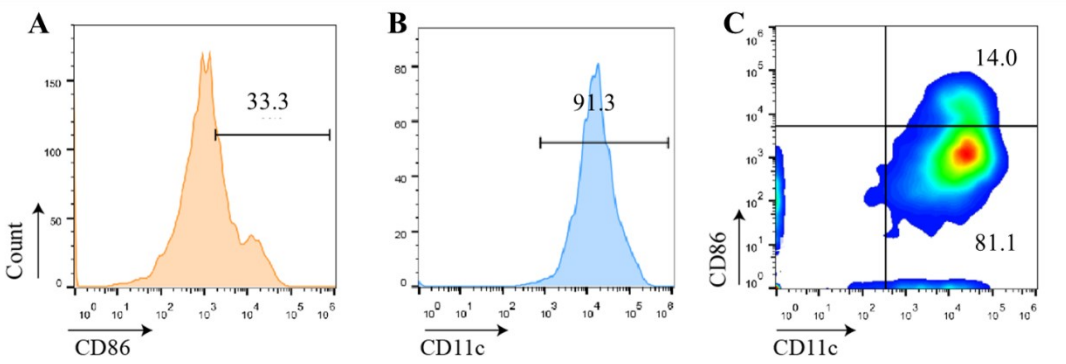

Figure S7. Determination the purity and maturity of BMDCs generated using Inaba method. (A) Purity of BMDCs. (B) Maturity of BMDCs. (C) Expression of CD11c and CD86 on BMDCs.

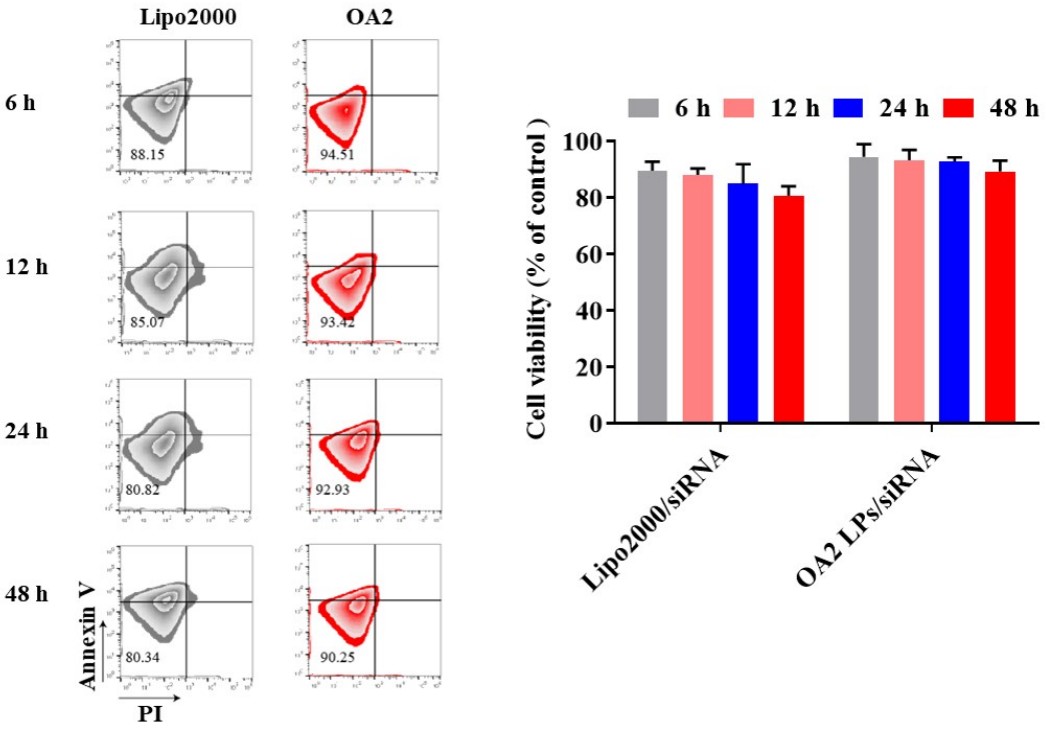

Figure S8. Apoptosis of BMDCs after incubation with lipo2000/siRNA and OA2 LPs/siRNA determined by the Annexin V-FITC/PI assay at 6 h, 12 h, 24 h, and 48 h (Mean ± SD, n = 3).

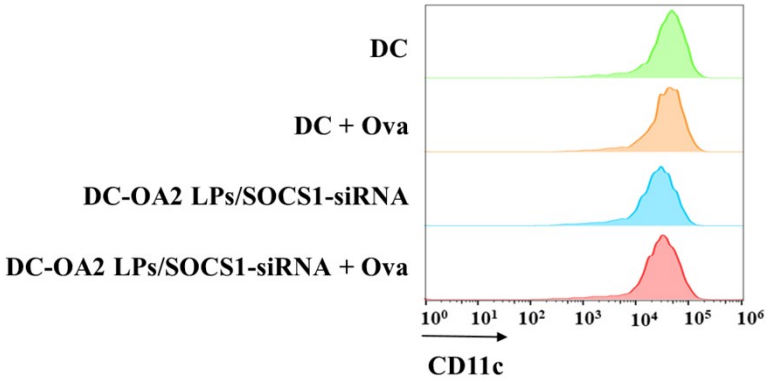

Figure S9. CD11c expression on BMCDs after incubating BMDCs with Ova, OA2 LPs/SOCS1-siRNA, and OA2 LPs/SOCS1-siRNA + Ova.

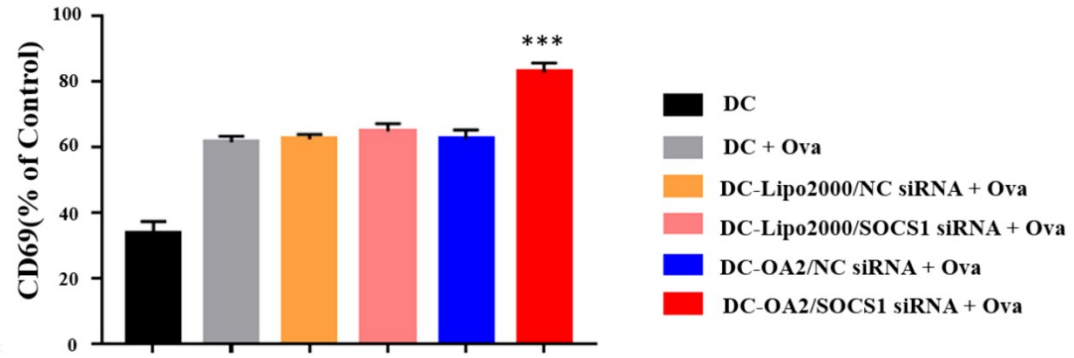

Figure S10. Quantification of CD69 expression in OT-1 T cells after 48 h-incubation of DC vaccines by flow cytometry (Mean ± SD, n = 3).

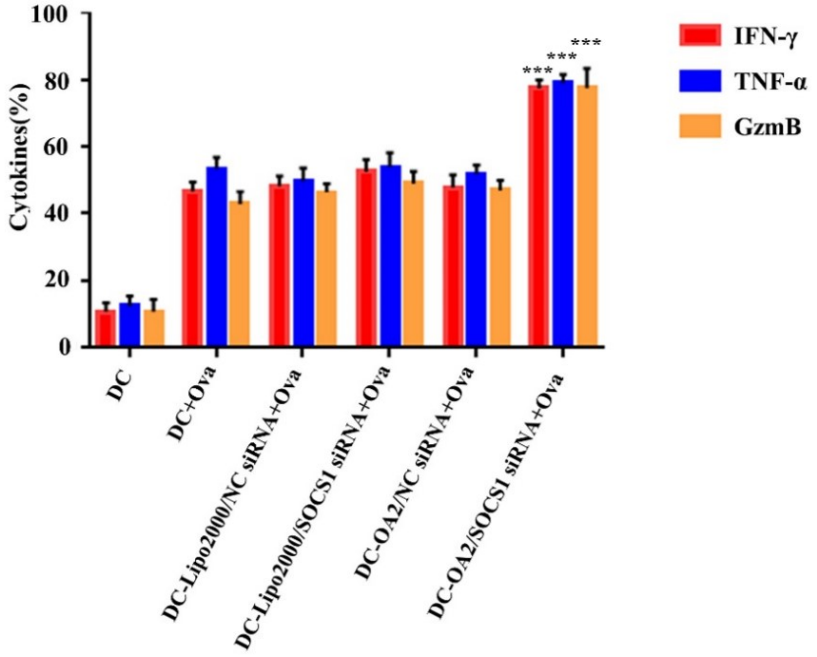

Figure S11. Quantification of intracellular IFN-γ, TNF-α and GzmB in OT-1 T cells after 48 h-incubation with different DC formulations (Mean ± SD, n=3).

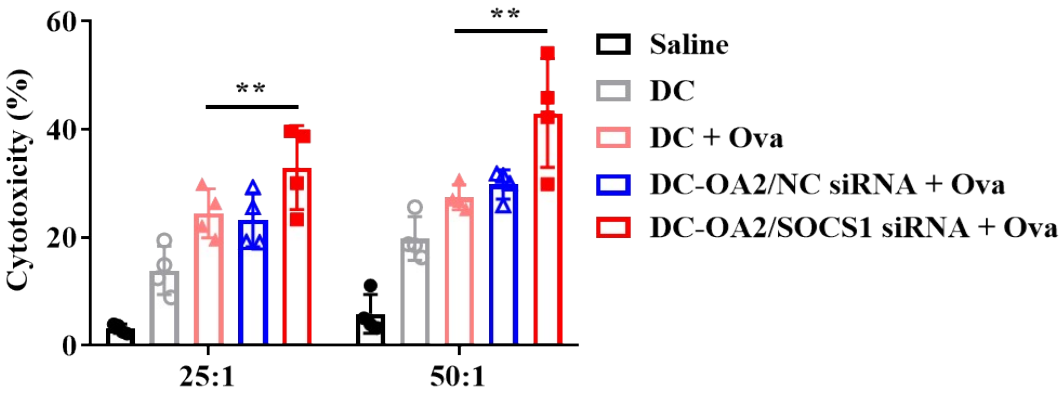

Figure S12. Cytotoxicity of spleen $CD8^+$ T cells against B16-Ova tumor cells as measured by release of lactate dehydrogenase (Mean ± SD, n = 4).

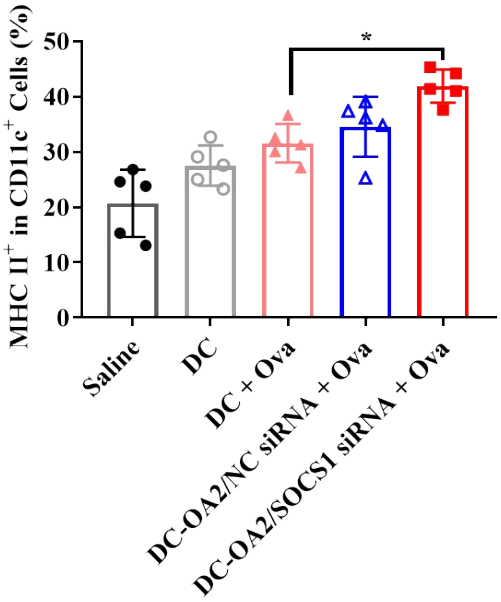

Figure S13. Quantitative analysis of tumor-infiltrated MHCII$^+$ DCs (Mean ± SD, n = 5).

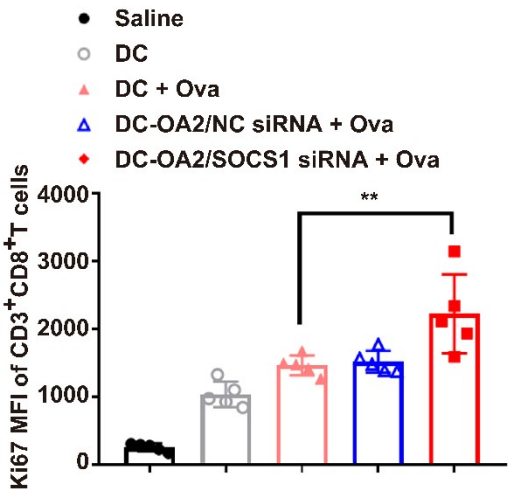

Figure S14. Quantitative analysis of Ki67 expression of tumor-infiltrated $CD3^+$ $CD8^+$ T cells. (Mean ± SD, n = 5).

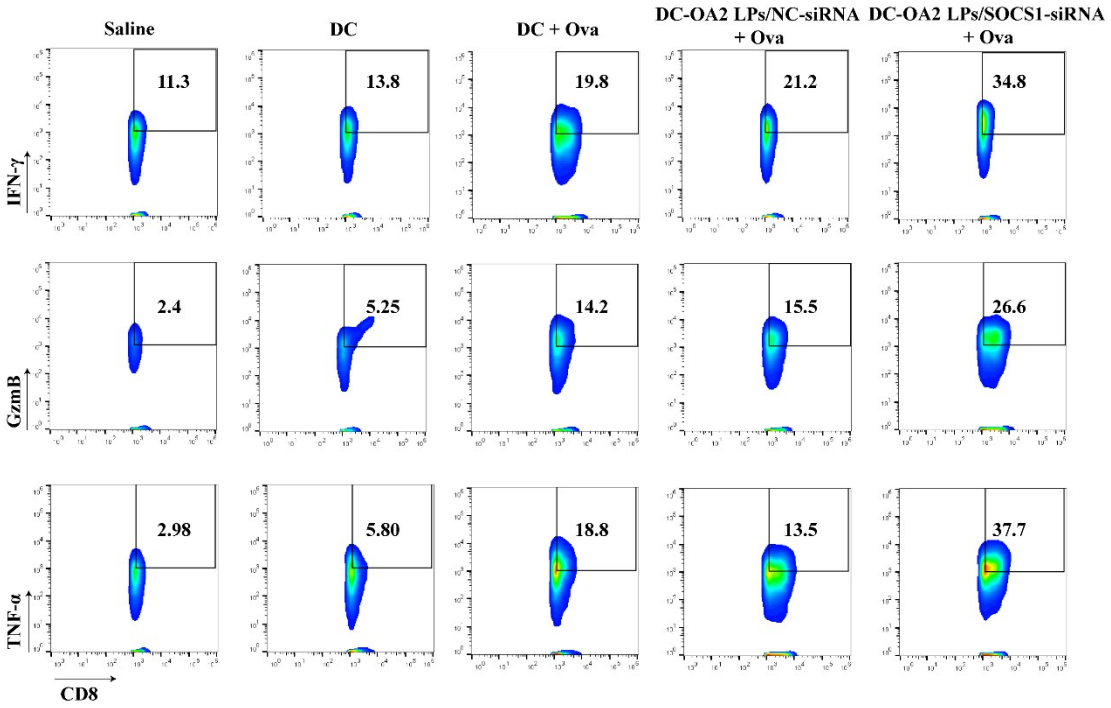

Figure S15. Representative flow cytometric images of IFN-γ, GzmB, and TNF-α expression in $CD3^+$ $CD8^+$ T cells isolated from the tumor tissue after treatment.

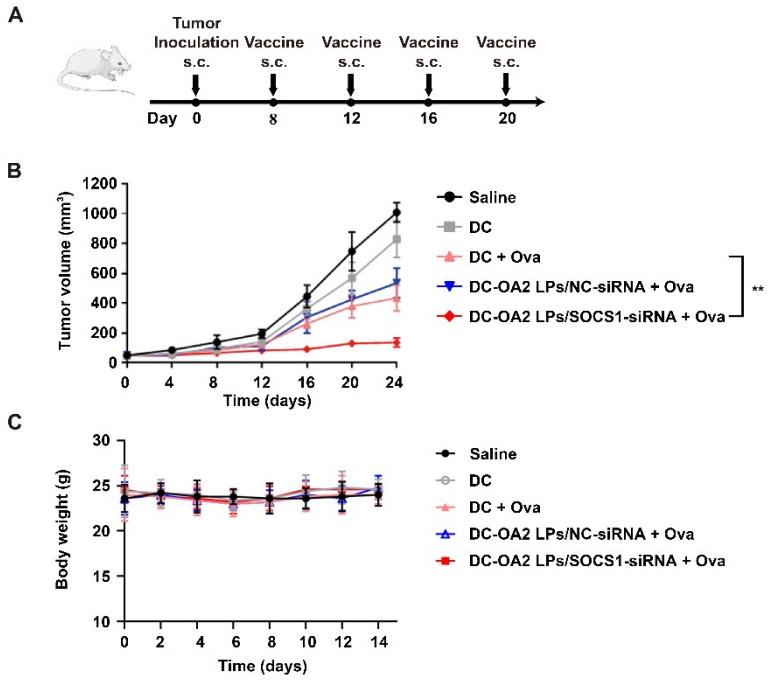

Figure S16. Tumor prevention assay. (A) Schematic illustration of the experimental design of tumor prevention. s.c., subcutaneous injection. (B) Tumor growth curves of immunized mice (Mean ± SD, n = 5). (C) Change in the body weight of the mice during the tumor prevention assay (Mean ± SD, n = 5).

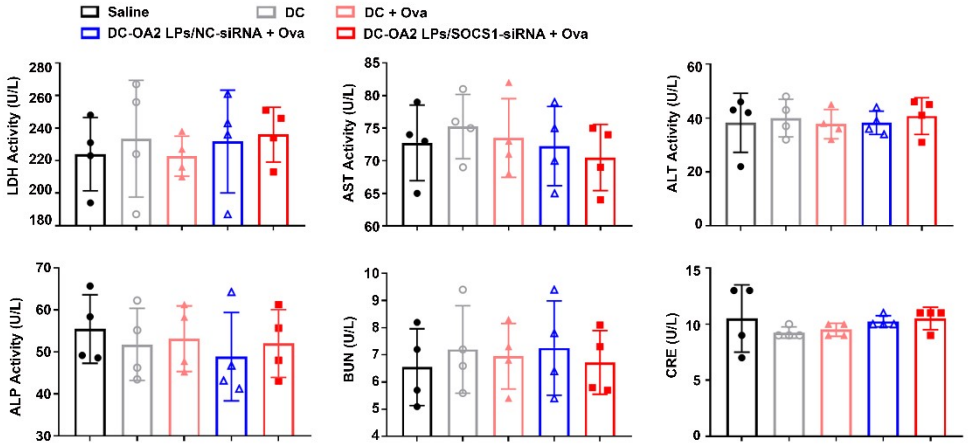

Figure S17. Quantification of LDH, AST, ALT, ALP, BUN and CRE in the plasma of the mice after treatment with indicated formulations (Mean ± SD, n = 4).

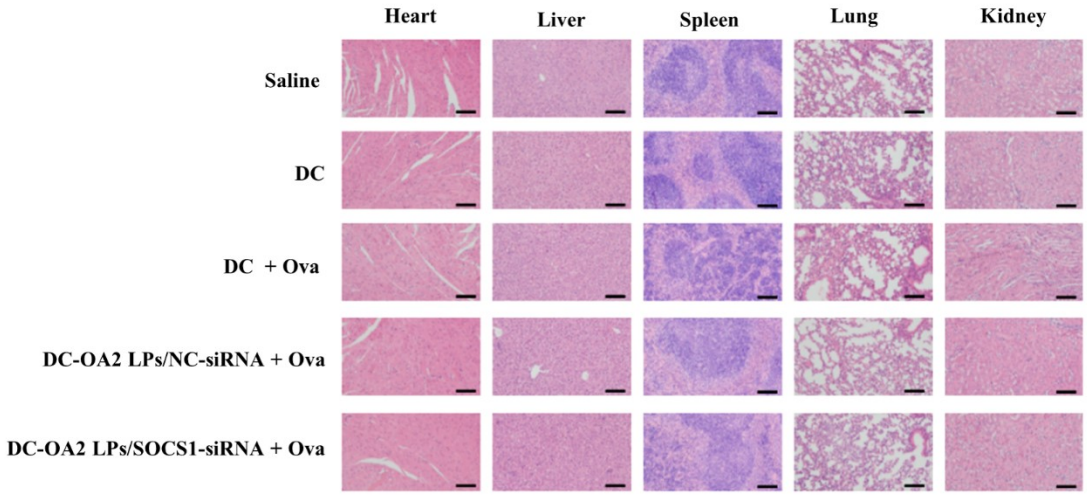

Figure S18. Histological images of the H&E-stained organs collected from the mice treated with indicated formulations. Scale bar: 100 μm.

Supporting Methods

Synthesis of OA2 lipid

1.1 Synthesis of OA2-Glu

A stirred solution of L-glutamic acid (5.00 g, 33.9 mmol) in toluene (200 mL) was added with *p*-toluenesulfonic acid (6.44 g, 37.4 mmol) followed by reflexing for 2 h at 140℃. Then, oleyl alcohol (19.2 g, 71.4 mmol) was added into the solution, followed by reflexing overnight at 150℃. The reaction mixture was evaporated with vacuum distillation to remove toluene and then dissolved in 300 mL Chloroform ($CHCl_3$). The organic phase was sequentially washed with saturated $NaHCO_3$ solution (200 mL × 2) and brine (200 mL × 1), and then dried over anhydrous Na_2SO_4, followed by filtration and concentration. The residue was purified by silica gel column chromatography eluting with petroleum ether: ethyl acetate = 10:1, and dried to obtain the compound as a colorless transparent oily liquid (6.40 g, 54% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 5.43-5.28 (m, 4H, $CH_2CHCHCH_2$), 4.17-4.01 (m, 4H, $COOCH_2$), 3.52-3.44 (m, 1H, NH_2CH), 2.46 (t, J = 7.2 Hz, 2H, CH_2CO), 2.09-1.93 (m, 8H, $CH_2CHCHCH_2$), 1.77-1.70 (m, 2H, NH_2CHCH_2), 1.69-1.55 (m, 4H, $COOCH_2CH_2$). 1.35-1.22 (m, 44H, $CH_{2(stearyl)}$), 0.88 (t, J = 6.7 Hz, 6H, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 175.05 (1C, CH_2COOCH_2), 172.70 (1C, NH_2CHCO), 129.47 (2C, $CH_2CHCHCH_2$), 129.25 (2C, $CH_2CHCHCH_2$), 64.73 (1C, $COOCH_2$), 64.19 (1C, $COOCH_2$), 53.26 (1C, NHCH), 31.40 (2C, $CH_2CH_2CH_3$), 30.13 (1C, CH_2COOCH_2), 29.26 (4C, $CH_2CHCHCH_2$), 29.23 (2C, $CH_{2(stearyl)}$), 29.19 (2C, $CH_{2(stearyl)}$). 29.02 (2C, $CH_{2(stearyl)}$), 28.91 (2C, $CH_{2(stearyl)}$), 28.82 (6C, $CH_{2(stearyl)}$), 28.72 (2C, $CH_{2(stearyl)}$), 28.09 (1C, $NHCHCH_2$), 26.71 (1C, OCH_2CH_2), 26.68 (1C, OCH_2CH_2), 25.40 (1C, $OCH_2CH_2CH_2$), 25.36 (1C, $OCH_2CH_2CH_2$), 22.18 (2C, CH_2CH_3), 13.61 (2C, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C_{41}H_{78}NO_4$ $[M+H]^+$, 648.5931; found, 648.5932.

1.2 Synthesis of OA2-Glu-Lys(Boc)$_2$

A stirred solution of Boc-Lys(Boc)-OH (484 mg, 2.085 mmol) in chloroform (30 mL) at 0℃ was added with EDCI (639 mg, 3.335 mmol) and HOBT (451 mg, 3.335

mmol) followed by stirring for 3 h at room temperature to obtain the reaction solution **A**. Next, a stirred solution of OA2-Glu (1.35 g, 2.085 mmol) in chloroform (20 mL) was added with triethylamine (872 μL, 6.254 mmol) followed by stirring for 1 h at room temperature to obtain the reaction solution **B**, which was slowly dripped into the reaction solution **A** to stir at room temperature overnight. The reaction mixture was sequentially washed with certain amount of water, 10% citric acid aqueous solution, and saturated brine, and then dried over anhydrous Na_2SO_4, followed by filtration and concentration. The residue was purified by silica gel column chromatography eluting with petroleum ether: ethyl acetate = 7:1, and dried to obtain the compound as a colorless transparent oily liquid (721 mg, 35.4% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.82 (brs, 1H, BocNH), 5.45-5.27 (m, 4H, $CH_2CHCHCH_2$), 5.16 (brs, 1H, BocNH), 4.71 (brs, 1H, BocNHCH), 4.63-4.55 (m, 1H, NHCH), 4.12 (t, J = 6.9 Hz, 2H, $COOCH_2$), 4.05 (t, J = 6.5 Hz, 2H, $COOCH_2$), 3.15-3.07 (brs, 2H, $NHCH_2$), 2.49-2.29 (m, 2H, CH2CO), 2.27-2.15 (m, 1H, $NHCHCH_2$), 2.06-1.92 (m, 8H, $CH_2CHCHCH_2$), 1.88-1.78 (m, 1H, $NHCHCH_2$), 1.73-1.54 (m, 4H, $COOCH_2CH_2$, 2H, $NHCH_2CH_2CH_2CH_2$), 1.46-1.42 (m, 18H, $C(CH_3)_3$, 2H, $NHCH_2CH_2$), 1.35-1.24 (m, 44H, $CH_{2(stearyl)}$, 2H, $NHCH_2CH_2CH_2$), 0.88 (t, J = 6.9 Hz, 6H, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 172.41 (1C, CH_2COOCH_2), 171.54 (1C, NHCHCO), 171.16 (1C, CHCONH), 155.66 (1C, $(CH_3)_3COCO$), 155.19 (1C, $(CH_3)_3COCO$), 129.47 (2C, $CH_2CHCHCH_2$), 129.26 (2C, $CH_2CHCHCH_2$), 94.60 (2C, $C(CH_3)_3$), 65.32 (1C, $COOCH_2$), 66.45 (1C, $COOCH_2$), 53.82 (1C, BocNHCH), 51.19 (1C, NHCH), 39.34 (1C, $NHCH_2$), 32.11 (1C, CH_2COOCH_2), 31.40 (2C, $CH_2CH_2CH_3$), 29.71 (1C, $NHCH_2CH_2$), 29.26 (4C, $CH_2CHCHCH_2$), 29.20 (1C, $CH_{2(stearyl)}$), 29.16 (1C, $CH_{2(stearyl)}$), 29.02 (2C, $CH_{2(stearyl)}$), 28.94 (2C, $CH_{2(stearyl)}$), 28.82 (8C, $CH_{2(stearyl)}$), 28.74 (2C, $CH_{2(stearyl)}$), 28.07 (1C, $NHCHCH_2$), 27.94 (3C, $(CH_3)_3C$), 27.80 (3C, $(CH_3)_3C$), 26.80 (1C, $NHCH_2CH_2CH_2CH_2$), 26.70 (2C, OCH_2CH_2), 25.39 (1C, $OCH_2CH_2CH_2$), 25.30 (1C, $OCH_2CH_2CH_2$), 22.18 (2C, CH_2CH_3), 21.95 (1C, $NHCH_2CH_2CH_2$), 13.62 (2C, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C_{57}H_{105}N_3O_9Na$ $[M+Na]^+$, 998.7749; found, 998.7747.

1.3 Synthesis of compound OA2

4.0 M HCl/1,4-dioxane solution (30 mL) was slowly dripped into the OA2-Glu-Lys $(Boc)_2$ (481 mg, 0.493 mmol) at 0℃. The reaction solution was concentrated and purified to obtain a yellow gelatinous solid with a yield of 80.2% (335 mg, 80.2% yield). ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.21 (brs, 2H, NH_2), 7.87 (brs, 2H, NH_2), 5.40-5.30 (m, 4H, $CH_2CHCHCH_2$), 4.55-4.39 (m, 1H, NH_2CH), 4.14-3.99 (m, 4H, $COOCH_2$), 3.27-3.08 (m, 1H, NH_2CH), 2.68-2.40 (m, 2H, NH_2CH_2), 2.19-2.10 (m, 2H, CH_2CO), 2.08-1.93 (m, 8H, $CH_2CHCHCH_2$, 2H, $NHCHCH_2$), 1.76-1.55 (m, 4H, $COOCH_2CH_2$, 2H, $NHCH_2CH_2CH_2CH_2$), 1.36-1.25 (m, 44H, $CH_{2(stearyl)}$, 2H, $NH_2CH_2CH_2CH_2$, 2H, $NH_2CH_2CH_2$), 0.88 (t, J = 7.1 Hz, 6H, CH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 173.32 (1C, CH_2COOCH_2), 171.44 (1C, NHCHCO), 169.47 (1C, CHCONH), 129.91 (2C, $CH_2CHCHCH_2$), 129.69 (2C, $CH_2CHCHCH_2$), 65.95 (1C, $COOCH_2$), 65.17 (1C, $COOCH_2$), 53.19 (1C, NH_2CH), 52.31 (1C, NHCH), 39.68 (1C, NH_2CH_2), 32.60 (1C, CH_2COOCH_2), 31.88 (2C, $CH_2CH_2CH_3$), 30.87 (1C, $NH_2CH_2CH_2CH_2CH_2$), 29.81 (2C, $CH_{2(stearyl)}$), 29.75 (4C, $CH_2CHCHCH_2$), 29.66 (2C, $CH_{2(stearyl)}$), 29.57 (2C, $CH_{2(stearyl)}$), 29.51 (2C, $CH_{2(stearyl)}$), 29.38 (2C, $CH_{2(stearyl)}$), 29.36 (2C, $CH_{2(stearyl)}$), 29.30 (2C, $CH_{2(stearyl)}$), 29.29 (2C, $CH_{2(stearyl)}$), 28.67 (1C, $NHCHCH_2$), 27.24 (1C, $NH_2CH_2CH_2$), 27.21 (2C, OCH_2CH_2), 25.99 (2C, $OCH_2CH_2CH_2$), 22.64 (2C, CH_2CH_3), 21.55 (1C, $NH_2CH_2CH_2CH_2$), 14.06 (2C, CH_2CH_3). HRMS, ESI^+, m/z: Calcd for $C47H90N3O5$ $[M+H]^+$, 776.6880; found, 776.6860.