Supporting Information for

M2-like tumor-associated macrophages-targeted codelivery of STAT6 inhibitor and IKKβ siRNA induces M2-to-M1 re-polarization for cancer immunotherapy with low immune side effects

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The supporting information includes:

MATERIALS AND METHODS

Chemicals and reagents. 2,5-dihydroxy-4-methyl-2,5-dioxy-3-furalopropionic acid, N-methoxycarbonyl maleimide were purchased from Aladdin (Shanghai, China). Methoxyl PEG-OH (Mn = 5 kDa) was purchased from Sigma-Aldrich (USA). AS1517499 (AS) was purchased from MedChem Express (Shanghai, China). M2peptide (YWWKVGWPDQEYC) and scrambled M2peptide (WGKYVPWQYDEWC) were purchased from Dechi Biosciences Co. Ltd. (Shanghai, China). Azadibenzocylooctyne-amine (DBCO-NH_2) was purchased from Biocone Biotechnology Co. Ltd. (Chengdou, China). Ethyl acetate, triethylamine (TEA), dichloromethane (CH_2Cl_2) and petroleum ether of analytical grade were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China) and dried over CaH_2 prior to use. All other reagents and solvents were used as obtained unless otherwise stated. 4',6'-diamidino-2-phenylindole (DAPI) was purchased from KeyGEN BioTECH Co. Ltd. (Nanjing, China). 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF, ultra-dry, with molecular sieves) were purchased from J&K Chemical Ltd. (Beijing, China). DMEM culture medium, RPMI-1640 culture medium, PBS, 0.25% trypsin-EDTA and fetal bovine serum (FBS) were purchased from ThermoFisher Scientific (Gibco, USA). Mouse IKKβ siRNA (sense: 5'-GGACAUCGUUGUUAGUGAATT-3', antisense: 5'-UUCACUAACAACGAUGUCCTT-3') was ordered from GenePharma Technology Co., Ltd. (Shanghai, China). D-luciferin, potassium salt was purchased from TEASEN Biotechnology Co., Ltd (Shanghai, China). The solid was dissolved in PBS at the concentration of 10 mg/mL and filtered through a 220 nm syringe filter, and the solution was stored at -20 ℃ prior to use.

The antibodies for flow cytometry analyses including anti-mouse CD45 (APC-Cy7 conjugated, Cat#103116), anti-mouse CD11b (PerCP-Cy5.5 conjugated, Cat#101228), anti-mouse F4/80 (FITC conjugated, Cat#123107), anti-mouse CD80 (APC conjugated, Cat#104714), anti-mouse CD206 (APC conjugated, Cat#141708), anti-mouse CD3 (APC conjugated, Cat#100236), anti-mouse CD8 (PE conjugated, Cat#100707), anti-mouse CD4 (FITC conjugated, Cat#100509), anti-mouse CD25 (BV421 conjugated, Cat#101923), anti-mouse Foxp3 (PE conjugated, Cat#126403) and anti-mouse IFN-γ (APC conjugated, Cat#505810) were purchased from BioLegend (San Diego, CA, USA). Other antibodies for western blotting, immunohistochemical staining and immunofluorescence staining including anti-IKKβ (ab171364, rabbit monoclonal antibody), anti-STAT6 (ab32520, rabbit monoclonal antibody), anti-STAT6 (phospho Y641) antibody (ab263947, rabbit monoclonal antibody), anti-iNOS (ab15323, rabbit polyclonal antibody), anti-Actin (ab8227, rabbit polyclonal antibody), anti-tubulin (ab7291, mouse monoclonal antibody), anti-CD206 (ab64693, rabbit polyclonal antibody), anti-CD80 (ab254579, rabbit polyclonal antibody), anti-CD8 (ab22378, rat monoclonal antibody), anti-Foxp3 (ab10901, rat polyclonal antibody), anti-IFN-γ (ab9657, rabbit polyclonal antibody), anti-TNF-α (ab225576, rabbit monoclonal antibody), goat anti-rabbit IgG Alexa Fluor® 488 (ab150077), goat anti-rabbit IgG Alexa Fluor® 647 (ab150079), goat anti-rat IgG Alexa Fluor® 488 (ab150157), goat anti-rat IgG Alexa Fluor® 555 (ab150158), goat anti-rat IgG Alexa Fluor® 647 (ab150159) were purchased from Abcam (Cambridge, UK). Secondary antibody for western blotting and immunohistochemical staining including goat anti-mouse IgG

antibody (HRP, A0216) and goat anti-Rabbit IgG antibody (HRP, A0208) were purchased from Beyotime Biotechnology (Shanghai, China). Anti-Arginase-1 antibody (#93668) for western blotting and immunohistochemical staining was purchased from Cell Signaling Technology (CST, USA).

Synthesis of azido-poly(N-benzyloxycarbonyl-L-lysine)-*b*-poly(β-benzyl-*L*-aspartate) (N_3-PBCLLys-PBLAsp). N-*ε*-benzyloxycarbonyl-*L*-lysine N-carboxyanhydride (BCLLys-NCA) and ε-benzyloxycarbonyl-*L*-aspartic acid N-carboxyanhydride (BLAsp-NCA) were synthesized as previously reported.[1,2] The diblock copolymer N_3-PBCLLys-PBLAsp was prepared by ring-opening polymerization of BCLLys-NCA and BLAsp-NCA.[3] Briefly, after azido-propylamine (18 mg, 0.18 mmol) was dissolved in 50 mL of anhydrous CH_2Cl_2, BCLLys-NCA (3.00 g, 10 mmol) dissolved in anhydrous DMF (5.0 mL) was added into the solution under N_2 atmosphere. The mixture was stirred at 35 °C for 48 h. N_3-PBCLLys was purified by precipitation into diethyl ether (1 L) for three times. The product was dried under vacuum to obtain a white solid. Finally, N_3-PBCLLys was used as a macromolecular initiator for the ring-opening polymerization of BLAsp-NCA to synthesize the diblock polymer N_3-PBCLLys-PBLAsp. Yield: 87.52%; Mn = 25.4 kDa as calculated from ^{1}H NMR spectrum.

Synthesis of azido-polylysine-*b*-poly(aspartic acid (N,N-diisopropylethylenediamine-*co*-benzylamine)) (N_3-PLys-PAsp(DIP-*co*-BZA)). Ammonolysis reaction of N_3-PBCLLys-PBLAsp was conducted as reported.[4] Briefly, N_3-PBCLLys-PBLAsp (1.52 g, 0.06 mmol) was dissolved in anhydrous DMSO (10 mL), and 0.10 g of benzylamine (1.0 mmol, 0.3 equiv. to the residual benzyl ester

groups in PBLAsp) was added into the solution. The reaction was kept at room temperature for 12 h before 0.72 g of N,N-diisopropylethylenediamine (4.95 mmol, 1.5 equiv. to the residual benzyl ester groups in PBLAsp) was added to react for another 12 h. The mixture was dialyzed (MWCO: 7.0 kDa) against methanol for 24 h and rotaevaporated to obtain the polymer N_3-PBCLLys-PAsp(DIP-*co*-BZA). Then, the N-benzyloxycarbonyl protection group was removed from N_3-PBCLLys-PAsp(DIP-*co*-BZA) using HBr/AcOH as reported.[5] In brief, N_3-PBCLLys-PAsp(DIP-*co*-BZA) (0.80 g, 0.03 mmol) was dissolved in 10 mL of acetic acid, and then 2 mL of 33 wt.% HBr/AcOH was added to react for 3 h at room temperature. The mixture was added into excessive anhydrous diethyl ether and the precipitate was collected. The solid was dissolved in pure water, dialyzed (MWCO: 7.0 kDa) against pure water for 48 h, and freeze-dried to obtain the white powder (N_3-PLys-PAsp(DIP-*co*-BZA)). Yield: 50.38%, Mn = 19.7 kDa as calculated from ^{1}H NMR spectrum.

Synthesis of TAMs-targeting peptide grafted copolymer (N_3-P[Lys(M2pep)-Lys]-PAsp(DIP-*co*-BZA)). N_3-PLys-PAsp(DIP-*co*-BZA) (0.40 g, 0.02 mmol) was dissolved in 5 mL of saturated $NaHCO_3$ solution cooled with ice bath. After 5.0 mg of N-methoxycarbonyl maleimide (0.03 mmol) (MCM) was added, the solution was stirred for 30 min and then reacted at room temperature for another 4 h. The solution was adjusted to pH 3.0 with 0.5 M hydrochloric acid, dialyzed against pure water, and freeze-dried. 0.20 g of the above solid was dissolved in 5 mL of dichloromethane, and then 32 mg of M2peptide (0.02 mmol) and 1.7 μL of TEA (12 μmol) were added. After reaction at room temperature for 24 h, dichloromethane was removed by rotary evaporation. The solid was dissolved in deionized water, dialyzed against deionized water (MWCO: 7.0 kDa) for 48 h, and then freeze-dried to get the powdery product.

Yield: 52.27%, Mn = 21.3 kDa as calculated from ^{1}H NMR spectrum.

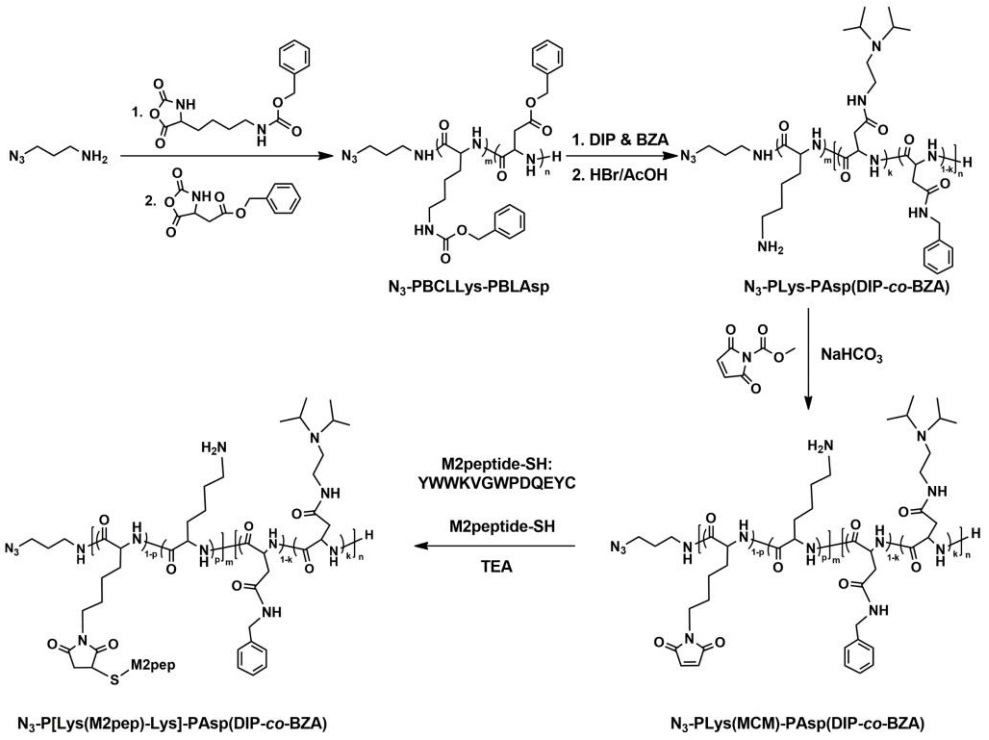
Synthesis of pH-sensitive methoxy-poly(ethylene glycol)-1-amide-2-propionic acid-3-methyl maleic acid-azadibenzocylooctyne-amine (mPEG$_{5k}$-phe-DBCO). According to the reported method,[6] 2, 5-dihydroxy-4-methyl-2, 5-dioxy-3-furalopropionic acid (0.276 g, 1.5 mmol) and 40 μL of DMF were added into 10 mL of anhydrous CH_2Cl_2 under N_2 atmosphere. After 0.378 g of oxalyl chloride (3 mmol) was dropwise added into the solution cooled in ice bath, the reaction was performed for 3 h at room temperature. The solvent CH_2Cl_2 and excessive oxalyl chloride were removed by rotary evaporation to obtain a yellow viscous liquid. 1.5 g of mPEG$_{5k}$-OH (0.2 mmol) dissolved in 10 mL of anhydrous CH_2Cl_2 was added to the above yellow viscous liquid. 30 μL of pyridine was added as a catalyst and the reaction was conducted at room temperature for 3 h. Then, the saturated NH_4Cl solution of the same volume was added to terminate the reaction. The organic phase was separated and dried with excessive anhydrous Na_2SO_4. The filtrate was precipitated into excessive anhydrous diethyl ether, and the precipitate was collected by centrifugation. The obtained solid (0.6 g, 0.12 mmol) was dissolved in 5 mL of CH_2Cl_2, and then 0.2 mL of DMF was added as catalyst. 63 mg of DBCO-NH_2 (0.24 mmol) was added into the solution for 24 h at room temperature, and then the solution was precipitated into excessive anhydrous diethyl ether and the precipitate (mPEG$_{5k}$-phe-DBCO) was collected by centrifugation.

Synthesis of pH-insensitive copolymer grafted with M2pep (mPEG-P[Lys(M2pep)-Lys]-PAsp(DIP-*co*-BZA)). mPEG$_{5k}$-NH_2 was synthesized from mPEG$_{5k}$-OH as reported.[7] mPEG$_{5k}$-NH_2 was used as a macromolecular initiator for ring-opening polymerization of BCLLys-NCA to synthesize methoxy-poly(ethylene

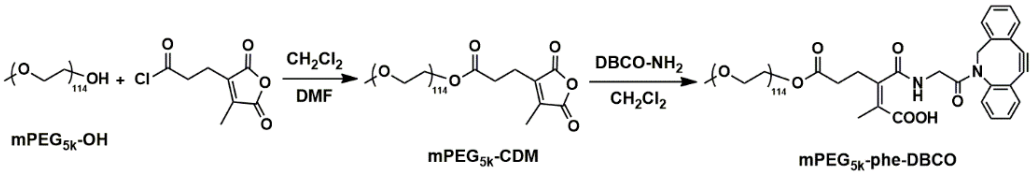
glycol)-poly(N-benzyloxycarbonyl-*L*-lysine) (mPEG-PBCLLys). Then, mPEG-PBCLLys was used as initiator for ring-opening polymerization of BLAsp-NCA to synthesize methoxy-poly(ethylene glycol)-poly(N-benzyloxycarbonyl-*L*-lysine)-*b*-poly(β-benzyl-*L*-aspartate) (mPEG-PBCLLys-PBLAsp). To obtain the polymer mPEG-PBCLLys-PAsp(DIP-*co*-BZA) *via* ammonolysis reaction, mPEG-PBCLLys-PBLAsp was treated with benzylamine (BZA) and N,N-diisopropylethylenediamine (DIP) in DMSO. Then, the N-benzyloxycarbonyl protection group was removed from mPEG-PBCLLys-PAsp(DIP-*co*-BZA) using HBr/AcOH to obtain the polymer mPEG-PLys-PAsp(DIP-*co*-BZA). M2pep was grafted onto mPEG-PLys-PAsp(DIP-*co*-BZA) to obtain the final product mPEG-P[Lys(M2pep)-Lys]-PAsp(DIP-*co*-BZA).

Polymer Characterization. ^{1}H NMR spectra of the polymers were recorded on a Bruker Biospin AVANCE III 400 MHz NMR spectrometer with DMSO-d_6 as a solvent. Fourier transform infrared (FTIR) spectral studies were carried out on a Thermo Nicolet Nexus 670 infrared spectrum analyzer at a resolution of 2 cm^{-1}.

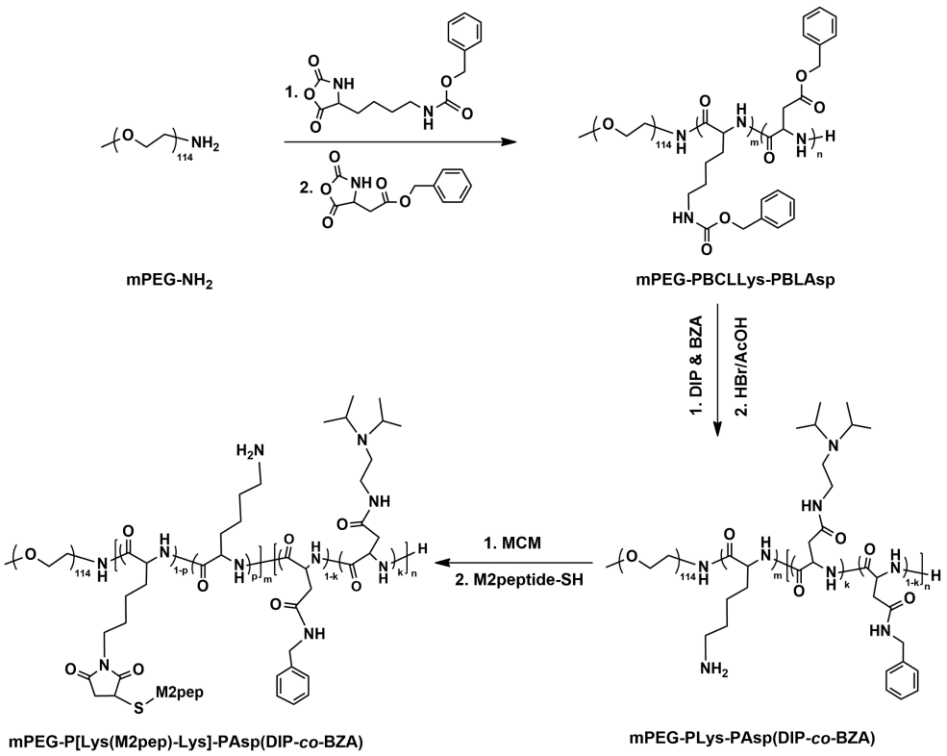
Supplemental Schemes



Scheme S1. Schematic illustration of synthetic approaches for TAMs-targeting peptide grafted diblock copolymer (N_3-P[Lys(M2pep)-Lys]-PAsp(DIP-*co*-BZA)).



Scheme S2. Synthetic approaches of mPEG-phe-DBCO designed to form pH-sheddable PEG corona on nanodrug.



mPEG-NH_2 mPEG-PBCLLys-PBLAsp

mPEG-P[Lys(M2pep)-Lys]-PAsp(DIP-*co*-BZA) mPEG-PLys-PAsp(DIP-*co*-BZA)

Scheme S3. Synthetic approaches of pH-insensitive TAMs-targeting peptide grafted triblock polymer mPEG-P[Lys(M2pep)-Lys]-PAsp(DIP-*co*-BZA).

Supplemental Figures

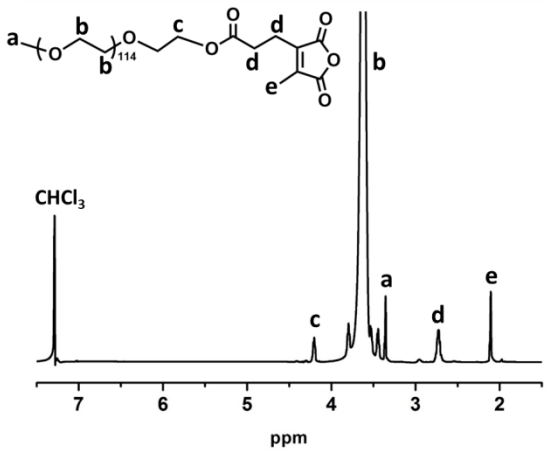


Figure S1. ^{1}H NMR spectra of mPEG$_{5k}$-CDM in $CDCl_3$. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) peaks at 3.35 ppm (-OC***H*$_3$**, a), 3.65 ppm (-OC***H*$_2$**C***H*$_2$**O- of PEG, b), 4.23 ppm (-CH_2C***H*$_2$**OCO-, c), 2.75 ppm (-COC***H*$_2$**C***H*$_2$**C(CO)=C-, d), 2.15 ppm (-C(CO)=CC***H*$_3$**, e).

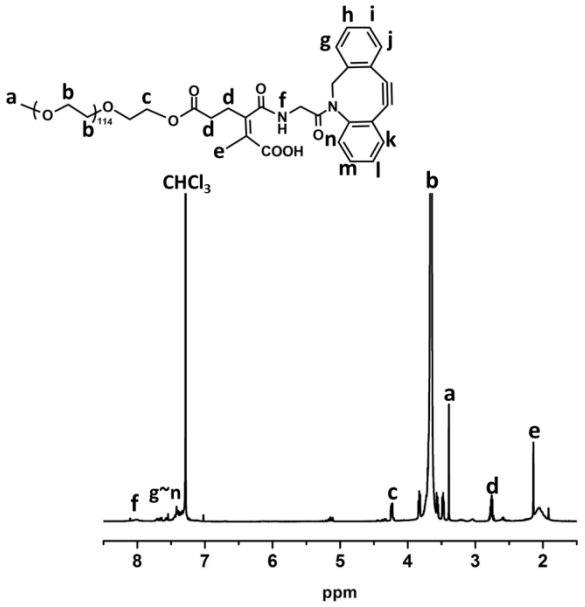


Figure S2. ^{1}H NMR spectra of mPEG$_{5k}$-phe-DBCO in $CDCl_3$. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) peaks at 3.35 ppm (-OC***H*****$_3$**, a), 3.65 ppm (-OC***H*****$_2$C***H*****$_2$**O- of PEG, b), 4.23 ppm (-CH_2C***H*****$_2$**OCO-, c), 2.75 ppm (-COC***H*****$_2$**C***H*****$_2$**C(CO)=C-, d), 2.15 ppm (-C(CO)=CC***H*****$_3$**, e), 7.30-7.70 ppm (g-n in DBCO), 7.95-8.05 ppm (-CON***H***CH_2-).

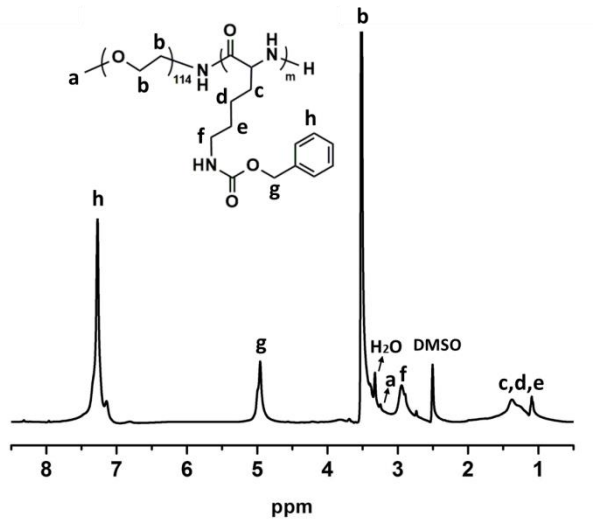


Figure S3. ^{1}H NMR spectra of mPEG-PBCLLys in DMSO-*d*$_6$. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 298 K) peaks at 3.30 ppm (-OC***H***$_3$ of PEG, a), 3.50 ppm (-OC***H***$_2$C***H***$_2$O- of PEG, b), 4.93-5.10 ppm (m, -C***H***$_2$C$_6$H$_5$ of PBCLLys, g), 1.24-2.05 ppm (-C***H***$_2$C***H***$_2$C***H***$_2$CH$_2$NHCO- of PBCLLys, c-e), 2.82-2.95 ppm (-C***H***$_2$NHCOO- of PBCLLys, f), 7.20-7.45 ppm (-CH$_2$C$_6$***H***$_5$ of PBCLLys, h). Polymerization degree of PBCLLys was calculated to be 55 according to integration of proton from methylene of PEG and benzyl groups of PBCLLys.

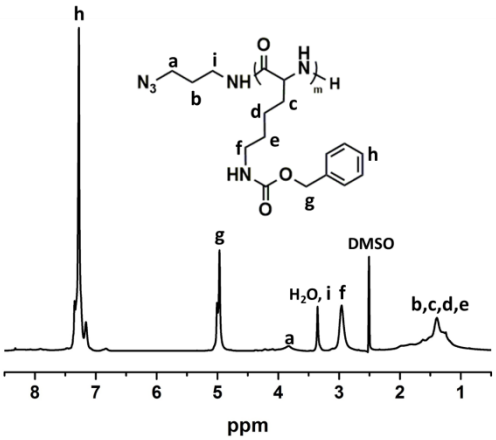


Figure S4. ^{1}H NMR spectra of N_3-PBCLLys in DMSO-d_6. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) peaks at 3.82 ppm ($N_3C\boldsymbol{H_2}$-, a), 1.01-2.10 ppm ($N_3CH_2C\boldsymbol{H_2}CH_2$-, -$C\boldsymbol{H_2}C\boldsymbol{H_2}C\boldsymbol{H_2}CH_2NHCO$- of PBCLLys, b-e), 2.82-2.95 ppm (-$C\boldsymbol{H_2}NHCOO$- of PBCLLys, f), 4.93-5.10 ppm (m, -$C\boldsymbol{H_2}C_6H_5$ of PBCLLys, g), 7.20-7.45 ppm (-$CH_2C_6\boldsymbol{H_5}$ of PBCLLys, h). Polymerization degree of PBCLLys was calculated to be 55 according to integration of proton from methylene of azidomethylene ($N_3C\boldsymbol{H_2}$-) and benzyl groups of PBCLLys.

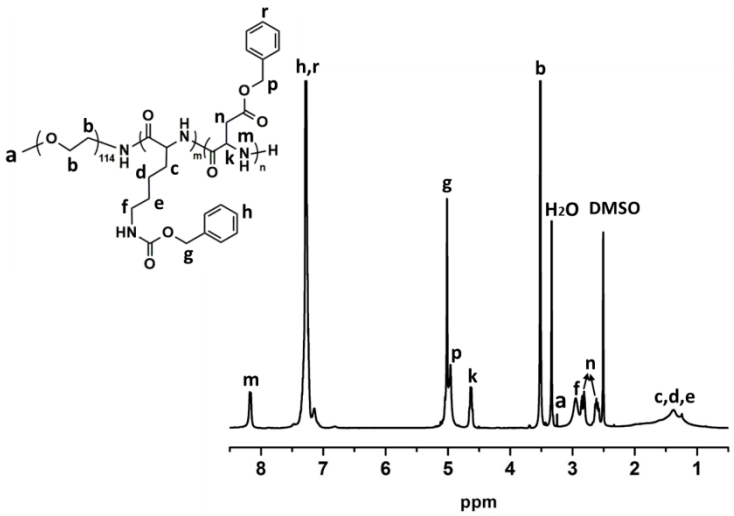


Figure S5. ^{1}H NMR spectra of mPEG-PBCLLys-PBLAsp in DMSO-*d*$_6$. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 298 K) peaks at 3.25 ppm (-OC***H***$_3$ of PEG, a), 3.50 ppm (-OC***H***$_2$C***H***$_2$O- of PEG, b), 1.01-2.10 ppm (-C***H***$_2$C***H***$_2$C***H***$_2$CH$_2$NHCO- of PBCLLys, c-e), 2.55-2.85ppm (-CH(C***H***$_2$COO-)NH- of PBLAsp), 2.85-2.95 ppm (-C***H***$_2$NHCO- of PBCLLys, f), 4.93-5.10 ppm (m, -C***H***$_2$C$_6$H$_5$ of PBCLLys and PBLAsp, g, p), 7.16-7.38 ppm (-CH$_2$C$_6$***H***$_5$ of PBCLLys and PBLAsp, h, r), 4.55-4.70 ppm (-C***H***(CH$_2$COO-)NH- of PBLAsp, k), 8.12-8.22 ppm (-CH(CH$_2$COO-)N***H***- of PBLAsp, m). Total polymerization degree of PBCLLys and PBLAsp was calculated to be 110 according to the integration of proton from methylene of PEG and benzyl groups of PBCLLys and PBLAsp.

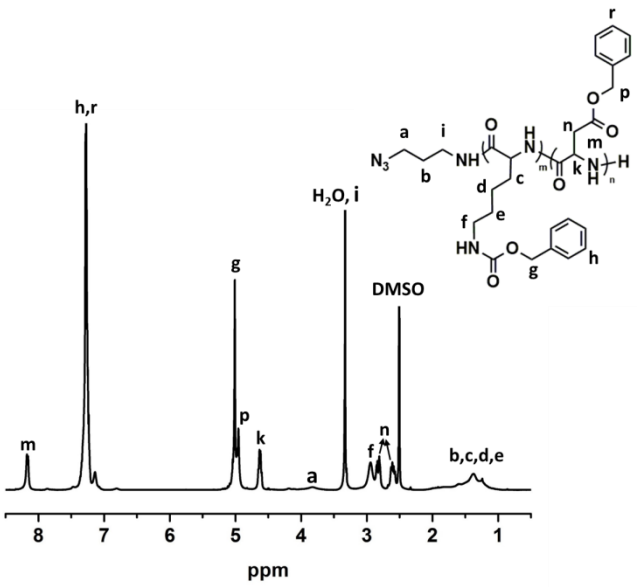


Figure S6. ^{1}H NMR spectra of N_3-PBCLLys-PBLAsp in DMSO-*d*$_6$. ^{1}H NMR (400 MHz, DMSO-*d*$_6$, 298 K) peaks at 3.82 ppm ($N_3C\boldsymbol{H_2}$-, a), 1.01-2.10 ppm ($N_3CH_2C\boldsymbol{H_2}CH_2$-, -$C\boldsymbol{H_2}C\boldsymbol{H_2}C\boldsymbol{H_2}CH_2NHCO$- of PBCLLys, b-e), 2.55-2.85ppm (-CH(C$\boldsymbol{H_2}$COO-)NH- of PBLAsp), 2.85-2.95 ppm (-C$\boldsymbol{H_2}$NHCO- of PBCLLys, f), 4.93-5.10 ppm (-C$\boldsymbol{H_2}C_6H_5$ of PBCLLys and PBLAsp, g, p), 7.20-7.40 ppm (-$CH_2C_6\boldsymbol{H_5}$ of PBCLLys and PBLAsp, h, r), 4.55-4.70ppm (-C$\boldsymbol{H}$(CH_2COO-)NH- of PBLAsp, k), 8.12-8.22 ppm (-CH(CH_2COO-)N$\boldsymbol{H}$- of PBLAsp, m). Total polymerization degree of PBCLLys and PBLAsp was calculated to be 110 according to the integration of proton from methylene of azidomethylene ($N_3C\boldsymbol{H_2}$-) and benzyl groups of PBCLLys and PBLAsp.

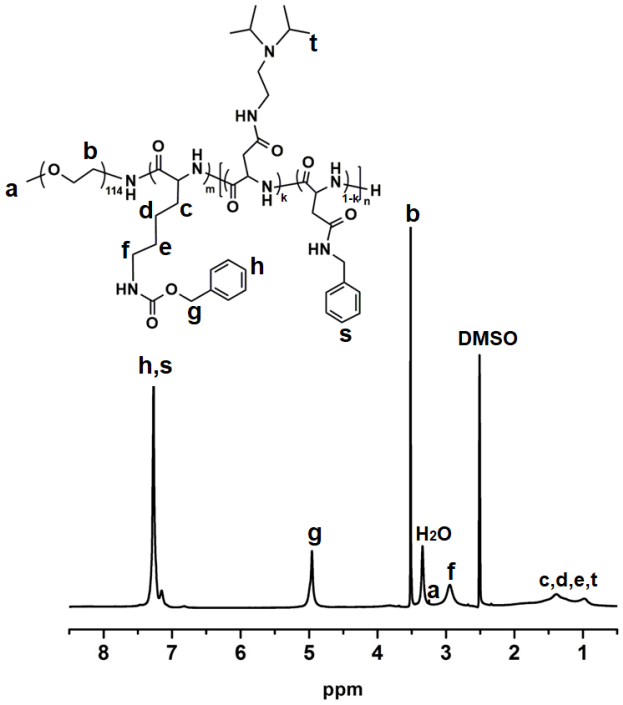


Figure S7. ^{1}H NMR spectra of mPEG-PBCLLys-PAsp(DIP-*co*-BZA) in DMSO-d_6. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) peaks at 3.25 ppm (-OC$\boldsymbol{H_3}$ of PEG, a), 3.62 ppm (-OC$\boldsymbol{H_2}$C$\boldsymbol{H_2}$O- of PEG, b), 1.01-2.10 ppm (-C$\boldsymbol{H_2}$C$\boldsymbol{H_2}$C$\boldsymbol{H_2}$CH$_2$NHCO- of PBCLLys, c-e, -N(CH(C$\boldsymbol{H_3}$)$_2$)$_2$ in DIP, t), 2.82-2.88 ppm (-C$\boldsymbol{H_2}$NHCO- of PBCLLys, f), 4.93-5.10 ppm (-C$\boldsymbol{H_2}$C$_6$H$_5$ of PBCLLys, g), 7.20-7.40 ppm (-CH$_2$C$_6$$\boldsymbol{H_5}$ of PBCLLys and PBLAsp(BZA), h, s).

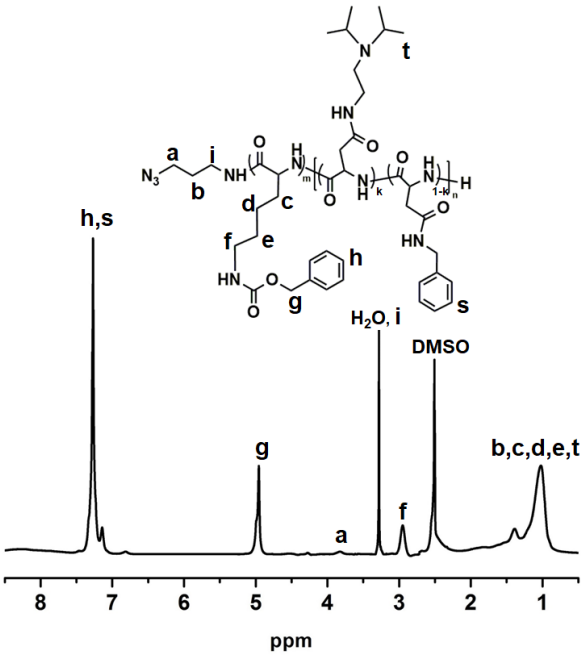


Figure S8. ^{1}H NMR spectra of N_3-PBCLLys-PAsp(DIP-*co*-BZA) in DMSO-d_6. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) peaks at 3.82 ppm (N_3CH_2-, a), 1.01-2.10 ppm ($N_3CH_2CH_2CH_2CH_2$-, -$CH_2CH_2CH_2CH_2NHCO$- of PBCLLys, b-e, -$N(CH(CH_3)_2)_2$ in DIP, t), 2.85-2.95 ppm (-CH_2NHCO- of PBCLLys, f), 4.93-5.10 ppm (-$CH_2C_6H_5$ of PBCLLys and PBLAsp, g, p), 7.20-7.40 ppm (-$CH_2C_6H_5$ of PBCLLys and PBLAsp(BZA), h, s).

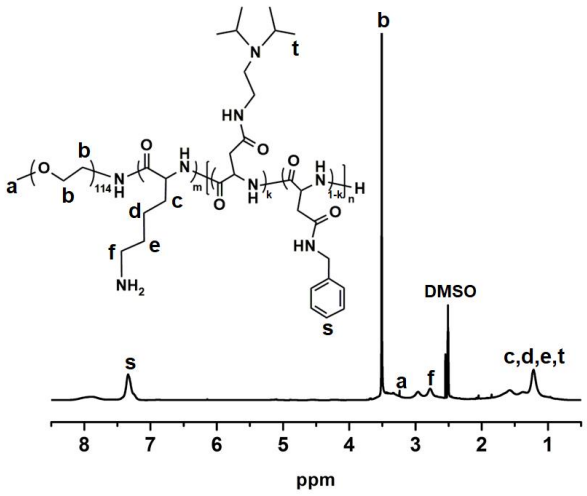


Figure S9. ^{1}H NMR spectra of mPEG-PLys-PAsp(DIP-*co*-BZA) in DMSO-d_6. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) peaks at 3.25 ppm (-OC$\boldsymbol{H_3}$ of PEG, a), 3.50 ppm (-OC$\boldsymbol{H_2}$C$\boldsymbol{H_2}$O- of PEG, b), 1.01-2.10 ppm (-C$\boldsymbol{H_2}$C$\boldsymbol{H_2}$C$\boldsymbol{H_2}$CH$_2$NHCO- of PBCLLys, c-e, -N(CH(C$\boldsymbol{H_3}$)$_2$)$_2$ in DIP, t), 2.65-2.85 ppm (-C$\boldsymbol{H_2}$NHCO- of PBCLLys, f), 7.16-7.38 ppm (-CH$_2$C$_6$$\boldsymbol{H_5}$ of PBLAsp(BZA), s).

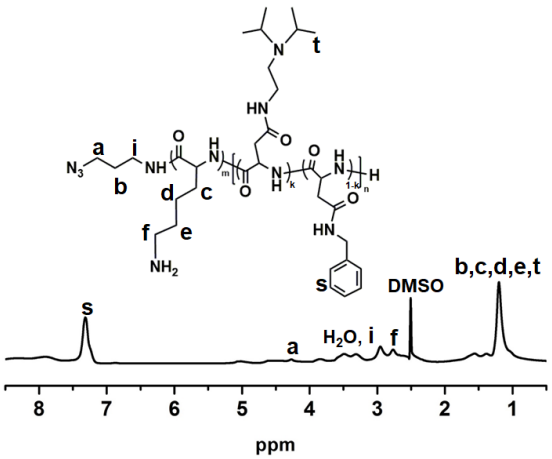


Figure S10. ^{1}H NMR spectra of N_3-PLys-PAsp(DIP-*co*-BZA) in DMSO-d_6. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) peaks at 3.82 ppm (N_3CH_2-, a), 1.01-2.10 ppm ($N_3CH_2CH_2CH_2CH_2$-, -$CH_2CH_2CH_2CH_2NHCO$- of PBCLLys, b-e, -$N(CH(CH_3)_2)_2$ in DIP, t), 2.65-2.85 ppm (-CH2NHCO- of PBCLLys, f), 7.20-7.40 ppm (-$CH_2C_6H_5$ of PBLAsp(BZA), h, s).

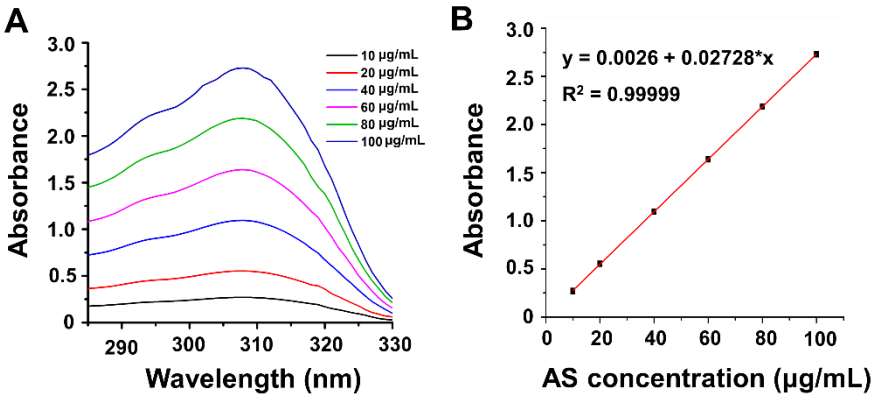


Figure S11. The measurement of drug loading content. (A) UV-Vis spectra of AS at various concentrations in DMSO. Characteristic peak of AS appears at 308 nm. (B) The standard curve of AS established through measuring absorbance at 308 nm with UV-Vis spectrophotometry.

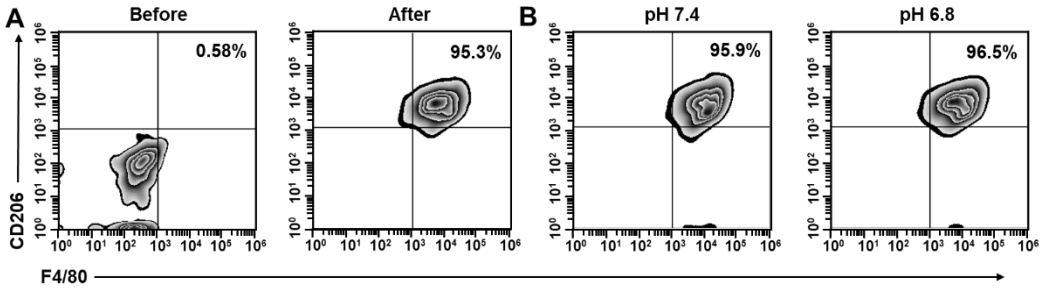


Figure S12. Representative flow cytometric analysis of the phenotype of the bone marrow derived macrophages. (A) Flow cytometric analysis of the bone marrow derived macrophages before and after treatment with MCS-F and IL-4. (B) Flow cytometric analysis of the phenotype of M2-like macrophages (CD206-positive) incubated at pH 7.4 or at pH 6.8. No significant difference was found between pH 6.8 and pH 7.4.

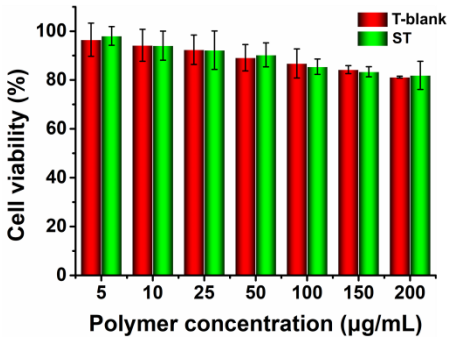


Figure S13. MTT assay showing viability of M2-like macrophages incubated with T-blank and ST for 24 h.

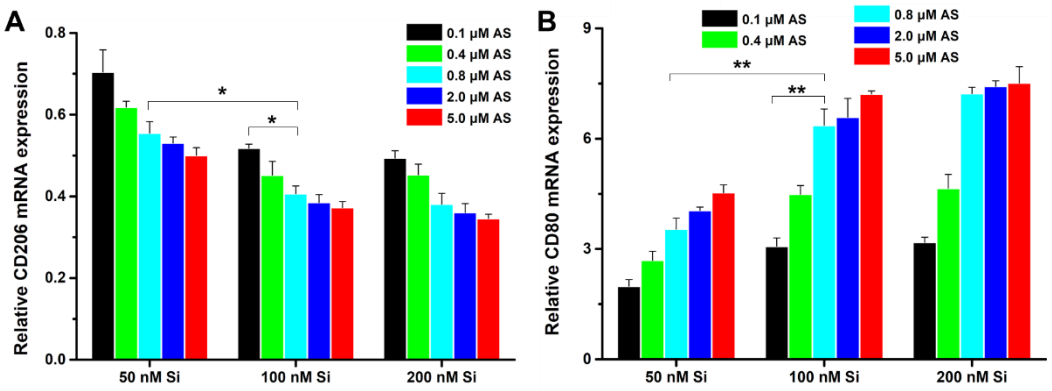


Figure S14. A dose-dependent macrophage repolarization of ST-AS&Si formulations. The mRNA levels of (A) CD206 and (B) CD80 genes determined by qRT-PCR in M2-like macrophages incubated with ST-AS&Si formulations at different AS/Si concentrations. $^{*}P < 0.05$, $^{**}P < 0.01$.

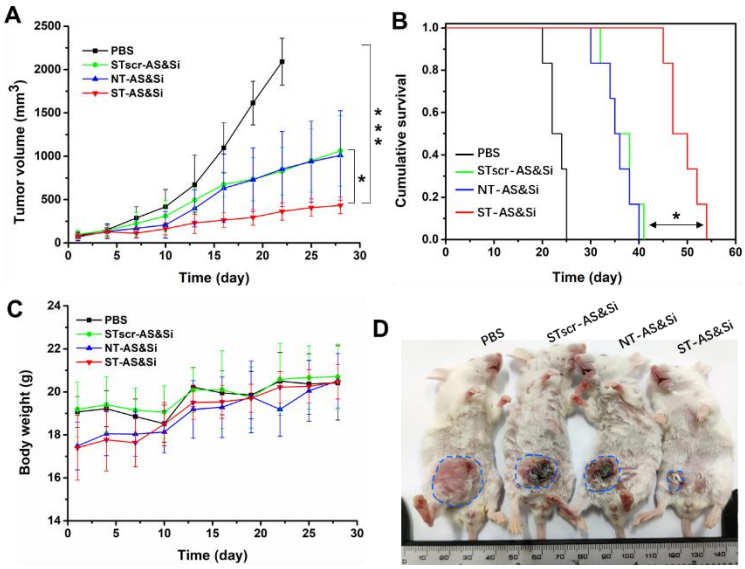


Figure S15. Antitumor immunotherapeutic effect in mice bearing 4T1 syngeneic orthotopic tumor. (A) 4T1 syngeneic orthotopic tumor growth in mice receiving different treatments. The injection was performed every three days for a total of seven treatments. (B) Cumulative survival of 4T1 tumor-bearing mice receiving different treatments. (C) The body weights of 4T1 syngeneic orthotopic tumor bearing mice receiving different treatments. (D) The representative photo of 4T1 tumor-bearing mice receiving different treatments. n = 6, $^{*}P < 0.05$; $^{***}P < 0.001$.

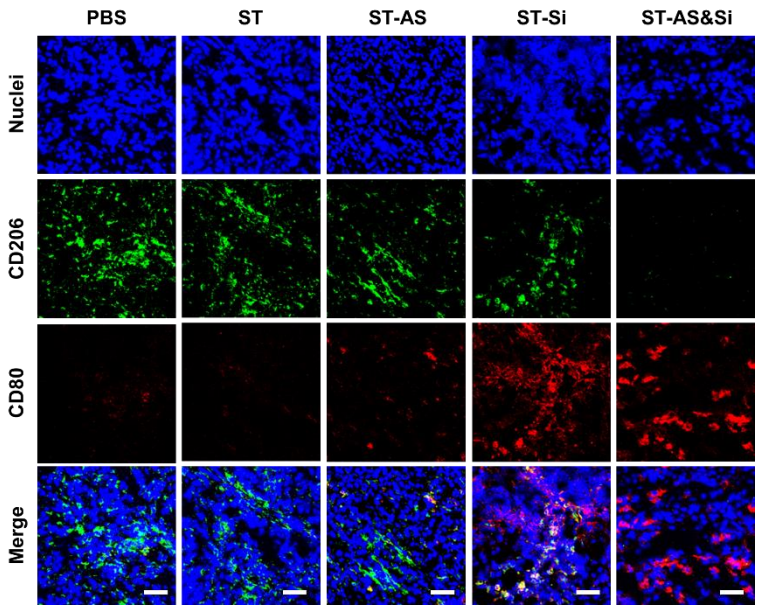


Figure S16. Immunofluorescent staining showing M2 and M1-like TAMs in tumor tissue of 4T1 tumor-bearing mice receiving various treatments. Scale bars represent 50 μm.

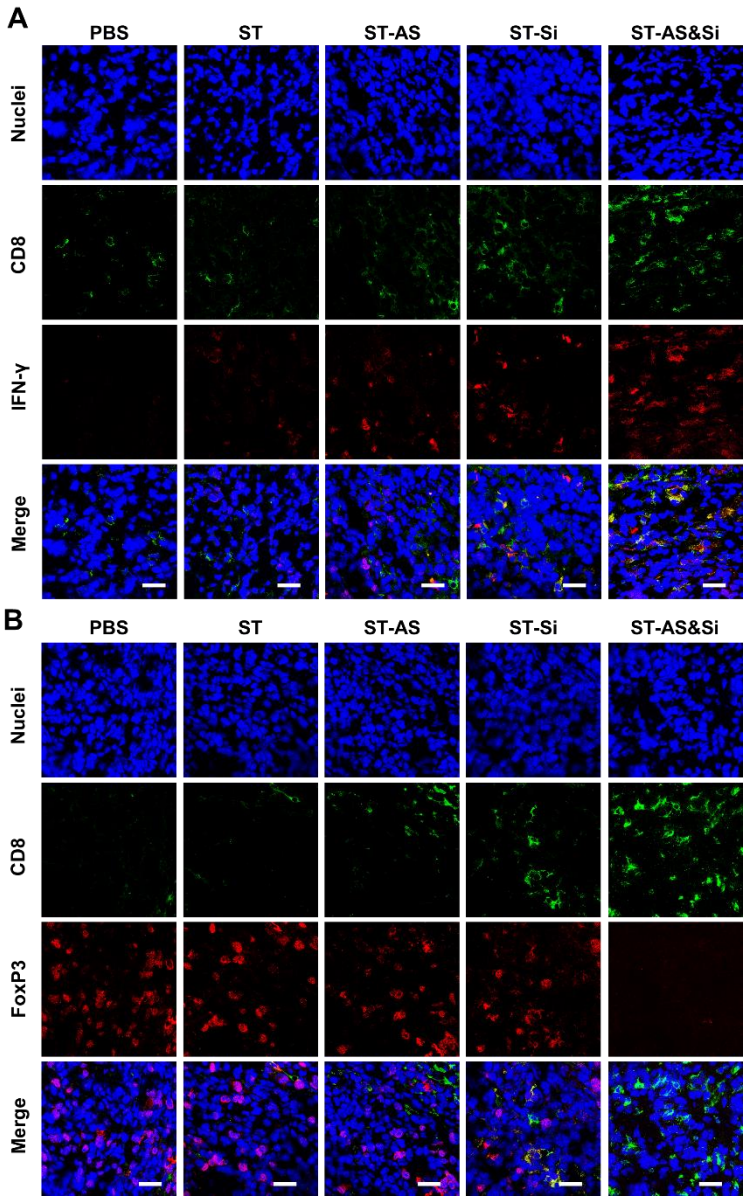


Figure S17. Immunofluorescent staining showing $CD8^+$ T cells and Th1 cells (A), $CD8^+$ T cells and Tregs (B) in tumor tissue of 4T1 tumor-bearing mice receiving various treatments. Scale bars represent 50 μm.

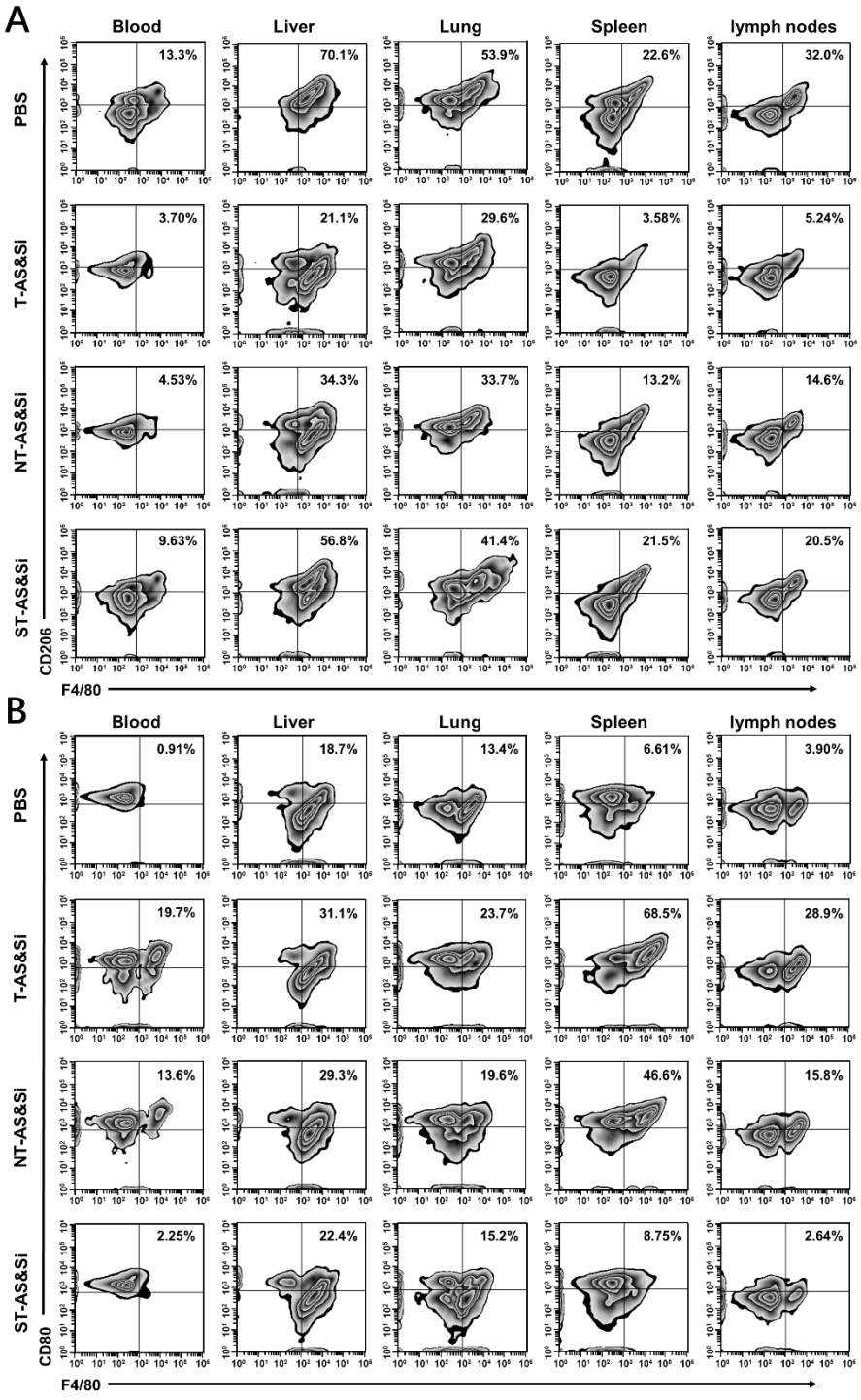


Figure S18. Re-polarization of macrophages in the mice receiving different formulations. Representative flow cytometric analysis displaying M2-like macrophages (A) and M1-like macrophages (B) in blood, liver, spleen, lung and lymph nodes. M1 and M2-like macrophages were gated on $CD45^+$ $CD11b^+$ $F4/80^+$ cells.

Supplemental Tables

Table S1. Characteristics of block copolymers.

Polymer	M_w (kDa)
N_3-PLL_{55}-$PBLA_{55}$	25.4
N_3-$PLys_{55}$-PAsp(DIP_{38}-*co*-BZA_{17})	19.7
N_3-P[Lys(M2pep)-Lys_{54}]-PAsp(DIP_{38}-*co*-BZA_{17})	21.3
mPEG-PLL_{55}-$PBLA_{55}$	30.4
mPEG-$PLys_{55}$-PAsp(DIP_{38}-*co*-BZA_{17})	24.7
mPEG-P[Lys(M2pep)-Lys_{54}]-PAsp(DIP_{38}-*co*-BZA_{17})	26.3

Calculated from H^1 NMR spectra.

Table S2. The forward and reverse sequences of gene primer.

gene	Forward (5'-3')	Reverse (5'-3')
IKKβ	GGCAGAAGAGCGAAGTGGACATC	CCAGCCGTTCAGCCAAGACAC
IL-10	CTGCTATGCTGCCTGCTCTTACTG	ATGTGGCTCTGGCCGACTGG
IL-12p70	CCTGTGACACGCCTGAAGAAGATG	CTTGTGGAGCAGCAGATGTGAGTG
Arginase I	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
TNF-α	GCGACGTGGAACTGGCAGAAG	GCCACAAGCAGGAATGAGAAGAGG
TGF-β	GCAACAATTCCTGGCGTTACCTTG	CAGCCACTGCCGTACAACTCC
IFN-γ	CAGGCCATCAGCAACAACATAAGC	AGCTGGTGGACCACTCGGATG
CD80	ACGACTCGCAACCACACCATTAAG	TGATGACAACGATGACGACGACTG
CD206	ACCTGGCAAGTATCCACAGCATTG	TGTTGTTCTCATGGCTTGGCTCTC
β-actin	CGAGCGTGGCTACAGCTTCA	AGGAAGAGGATGCGGCAGTG

Table S3. The table of serum levels of blood urea nitrogen (BUN), creatinine (CRE), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in 4T1 tumors-bearing mice receiving different treatments (n = 6).

	ALT (U/L)	AST (U/L)	BUN (mmol/L)	CRE (μmol/L)
Reference range	27 ~ 195	43 ~ 397	5 ~ 26	15 ~ 88
PBS	32.14 ± 7.64	243.33 ± 37.85	8.29 ± 1.38	17.81 ± 1.05
STscr-AS&Si	33.70 ± 16.35	190.66 ± 45.81	6.51 ± 1.46	17.62 ± 0.73
NT-AS&Si	28.21 ± 7.02	180.35 ± 52.78	5.92 ± 1.07	17.06 ± 1.24
ST-AS&Si	31.59 ± 3.64	185.56 ± 49.93	5.57 ± 0.69	16.73 ± 1.56

Reference

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