Supplementary data for "Guanidinylated cationic nanoparticles as robust protein antigen delivery systems and adjuvants for promoting antigen-specific immune responses in vivo"

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PEDG copolymer synthesis and charaterization

1. Synthesis of mPEG-*b*-PDPA-*b*-PGEM

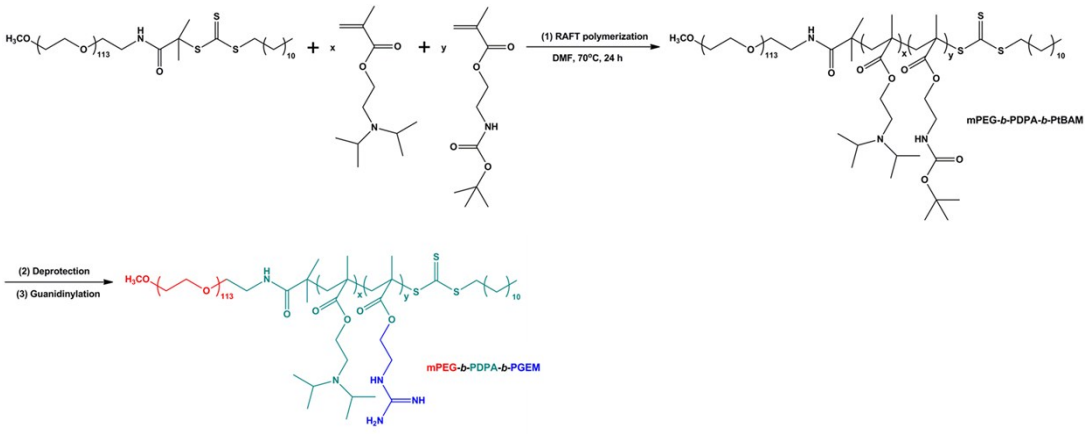


Fig. S1 The synthesis route of PEDG triblock copolymer.

1.1 Synthesis of mPEG-*b*-PDPA-*b*-PtBAM

Monomethoxy poly (ethylene glycol)-*block*-poly(2-(diisopropyl amino) ethyl methacrylate)-

block- poly(N-(tert-butoxycarbonyl) amino ethyl methacrylate) (mPEG-*b*-PDPA-*b*-PtBAM) was synthesized via the sequential RAFT polymerization of DPA and tBAM monomers using mPEG-CTAm as the chain transfer agent. The synthesis route was shown in Fig. S1. Briefly, mPEG-CTAm (0.1 mmol, 500 mg), DPA (5.0 mmol, 1065 mg) and AIBN (0.02 mmol, 3.0 mg) were added to a Schlenk tube and dissolved in anhydrous DMF (3 mL). The mixture was degassed by three cycles of freeze-pump-thaw and heated at 70 ^{o}C for 24 h in argon atmosphere. Then, tBAM (3.0 mmol, 653.5 mg) and AIBN (0.02 mmol, 3.0 mg) were added into the tube and the polymerization was continued for another 24 h. The resulted solution was sealed in a dialysis bag with a MWCO of 3.5 kDa, dialyzed against ultra-pure water for two days, and then lyophilized to obtain the powder of mPEG-*b*-PDPA-*b*-PtBAM copolymers.

1.2 Synthesis of mPEG-*b*-PDPA-*b*-PGEM

Monomethoxy poly (ethylene glycol)-*block*-poly(2-(diisopropyl amino) ethyl methacrylate)-*block*- poly(2-(guanidyl) ethyl methacrylate) (mPEG-*b*-PDPA-*b*-PGEM) was obtained by the deprotection of Boc groups on mPEG-*b*-PDPA-*b*-PtBAM and the following guanidinylation of primary amines of the deprotection products. mPEG-*b*-PDPA-*b*-PtBAM copolymer (1.0 g) was dissolved in trifluoroacetic acid (TFA, 5 mL) and stirred for 2 h at room temperature. TFA was removed by rotary evaporation and the residue was dissolved in DMF. The resultant solution was then precipitated in diethyl ether and the precipate was collected by filtration, washed twice by diethyl ether, and dried overnight under vacuum. Finally, the primary amines of the obtained monomethoxy poly (ethylene glycol)-*block*-poly(2-(diisopropyl amino) ethyl methacrylate)-*block*-poly(2-aminoethyl methacrylate) (mPEG-*b*-PDPA-*b*-PAEM) were

guanidinylated in 0.1 M $NaHCO_3$ solution. After addition of 2 equivalent moles of *S*-ethylisothiourea hydrobromide, the mixture was stirred for two days at room temperature. Afterwards, the reaction mixture was placed into a dialysis bag with MWCO of 3.5 kDa and dialyzed against ultra-pure water for 3 days. The final monomethoxy poly(ethylene glycol)-*block*-poly(2-(diisopropyl amino) ethyl methacrylate)-*block*-poly(2-(guanidyl) ethyl methacrylate) (mPEG-*b*-PDPA-*b*-PGEM, PEDG) polymer was recovered by lyophilization.

1.3 Characterization of polymers

^{1}H NMR and ^{13}C NMR spectrum of mPEG-*b*-PDPA-*b*-PGEM were acquired on a Varian Unity spectrometer (500 MHz) using the mixture of D_2O/phosphoric acid-d_3 (v/v=60/1) as the solvent. The molecular weight distribution of mPEG-*b*-PDPA-*b*-PGEM was performed by the gel permeation chromatography (GPC) system, which was equipped with a CoMetre 6000 LDI pump, a Schambeck SFD GmbH RI2000 refractive index detector and a ShodexOHpak SB-802.5 column. The mobile phase was composed of 0.3 M NaH_2PO_4 and 1.0 M acetic acid in water. The flow rate is 0.5 mL/min and the detection temperature was 40 ^{o}C. All samples were filtered through a 0.22 mm filter before analysis and poly (methyl methacrylate) was used as the standard.

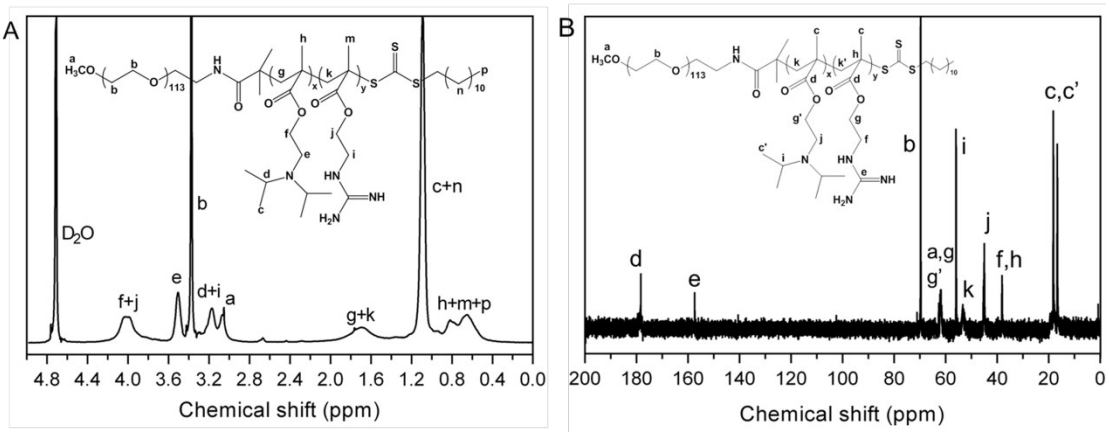


Fig. S2 ^{1}H NMR (A) and ^{13}C NMR (B) spectra of PEDG copolymer.

1.4 Determination of the critical micelle concentration (CMC) of PEDG copolymer

Pyrene was used as the probe for CMC determination. The PEDG solution containing pyrene was prepared as follows. Briefly, a known amount of pyrene in THF was added to each of 10 mL vials and then THF was evaporated. A total of 10 ml of copolymer aqueous solutions with various concentrations were added to each vial and equilibrated for 24 h at room temperature. The copolymer concentration in this experiment varied from 1.0 x 10^{-6} to 1 mg/mL. The pyrene concentration in copolymer solution was 6 x 10^{-7} M. Fluorescence intensities of the pyrene were determined by a Varian fluorescence spectrophotometer at room temperature. For the measurement of pyrene excitation spectra, the emission wavelength was set at 373 nm with the scan speed of 240 nm/min and the slit width of 10 nm. The ratio between the fluorescence intensity of emission wavelength at 337 nm and the fluorescence intensity of that at 330 nm was calculated and I_{337}/I_{333} values verse logarithmic polymer concentration was fitted to sigmoidal curve (Fig. S3). The IC50 value was considered as the CMC.

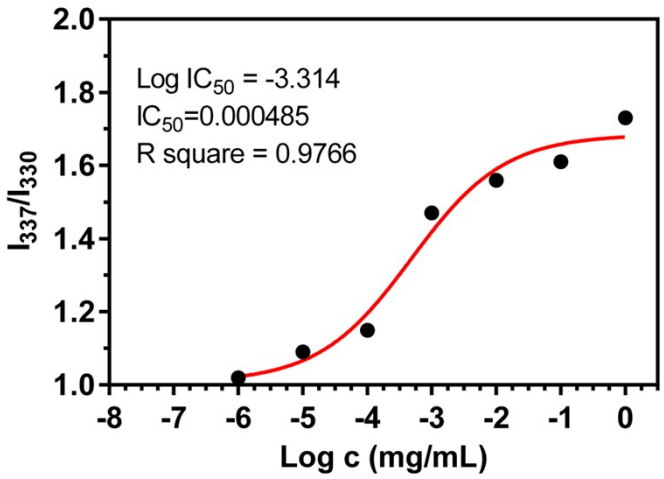


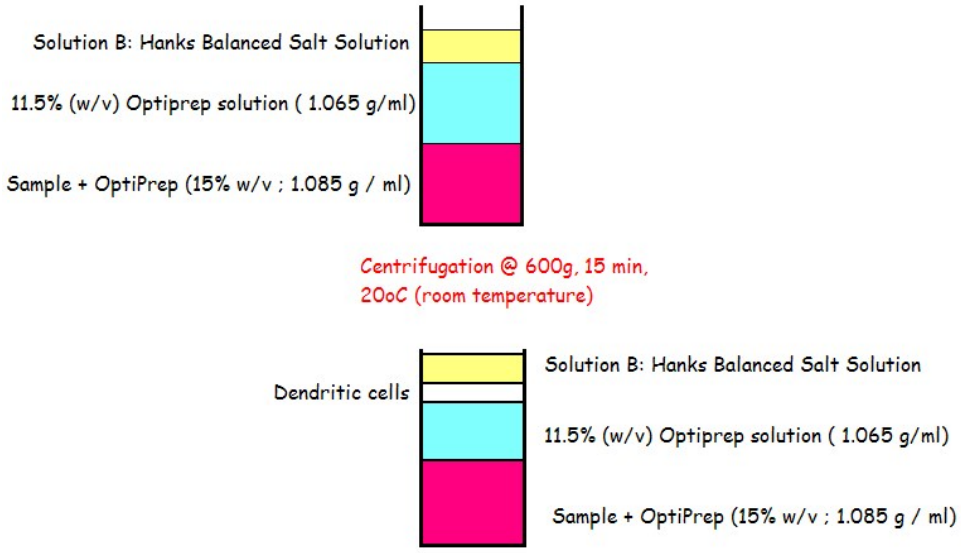
Fig. S3 The I_{337}/I_{330} values as function of logarithmic polymer concentration. The red line is the sigmoidal fitting curve.

1.5 Isolation of mouse dendritic cells by flotation through a low density barrier

Materials: Optiprep™ Density Gradient Medium were purchased from Sigma-ALDRICH® (Catalog number: D1556, Shanghai ,China). Hanks Balanced Salt solution without Ca^{2+} and Mg^{2+} were from Gibco® life technologies (Catalog number: 14175103, Beijing China). HEPES buffer saline was acquired from Sigma-ALDRICH® (Catalog number: 51558, Shanghai, China).

Solution preparation: A, Optiprep™ Density Gradient Medium; B, Hanks Balanced Salt solution without Ca^{2+} and Mg^{2+}; C, Diluent: 0.88% (w/v) NaCl (sodium chloride), 1mM EDTA, 0.5% (w/v) bovine serum albumin (BSA), 10mM Hepes-NaOH, pH 7.4. Harvest cells by centrifugation (500*g*, 20 min) and resuspend and wash in 3 mL of HBSS buffer, mix washed cells gently but thoroughly with OptiPrep (3:1 v/v ie 3 mL cells + 1 mL OptiPrep). Add 4 mL of the suspension to 5 mL of 11.5% (w/v) density gradient (made from OptiPrep and Solution C at 1:4.2 v/v) and 3 mL solution B. Centrifuge at 600 g for 15 min at 20 ^{o}C,

allow the rotor to decelerate without the break (acceleration/deceleration speed 1) and harvest DCs from the top of the 11.5% iodixanol layer (Scheme S1).



Scheme S1 Separation and purification of dendritic cells by flotation through a low density barrier.

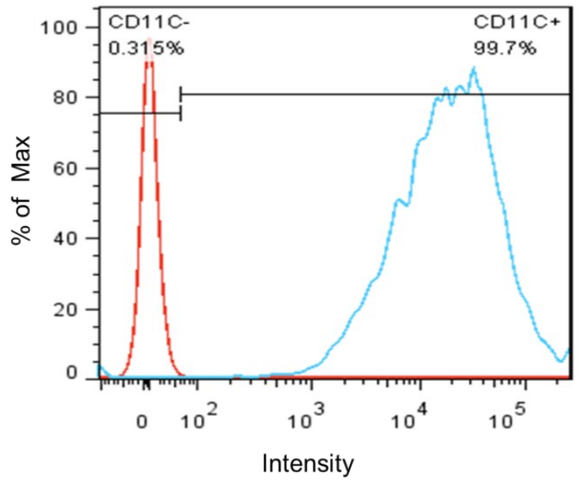


Fig. S4 The flow cytometry profile of BMDCs isolated by flotation.

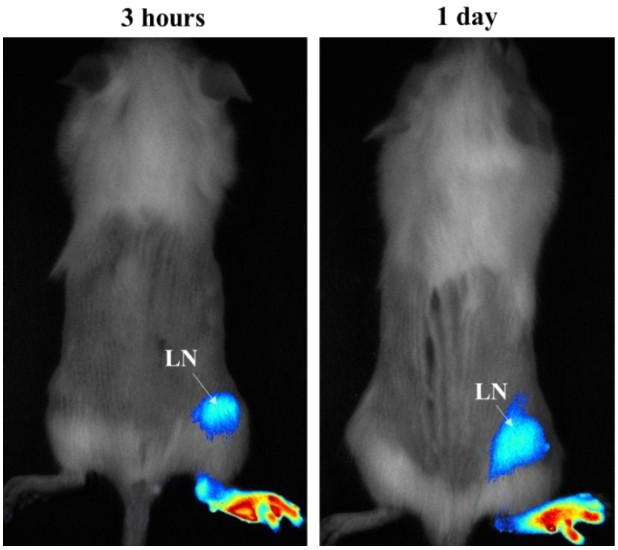


Fig. S5 In vivo visualization of antigens at the footpad injection site and draining lymph nodes. RBITC-conjugated OVA was encapsulated in PEDG nanoparticles and the injection dose is 20 μg with an injection volume of 100 μL.

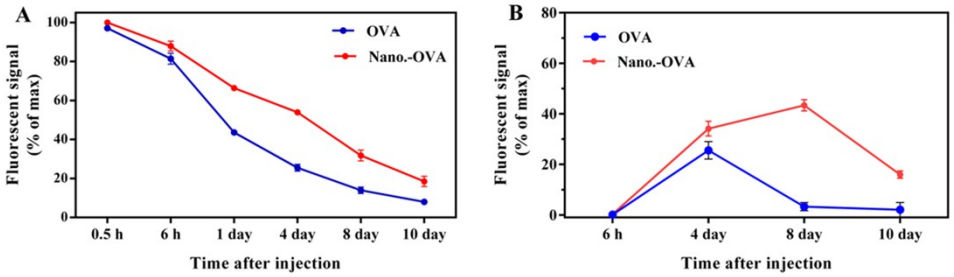


Fig. S6 Quantification of the fluorescence signals of RBITC-conjugated OVA at the tail-based site (A) and at the draining lymph nodes (B). Data was expressed as mean ± SD (n=3).