Negative regulation of cationic nanoparticle-induced inflammatory toxicity through the increased production of prostaglandin E2 via mitochondrial DNA-activated Ly6C$^+$ monocytes

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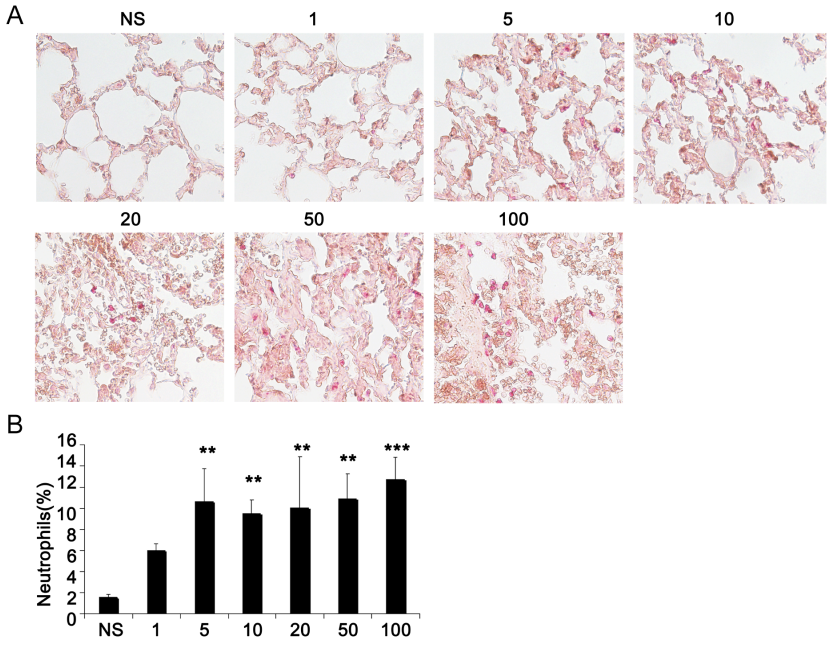
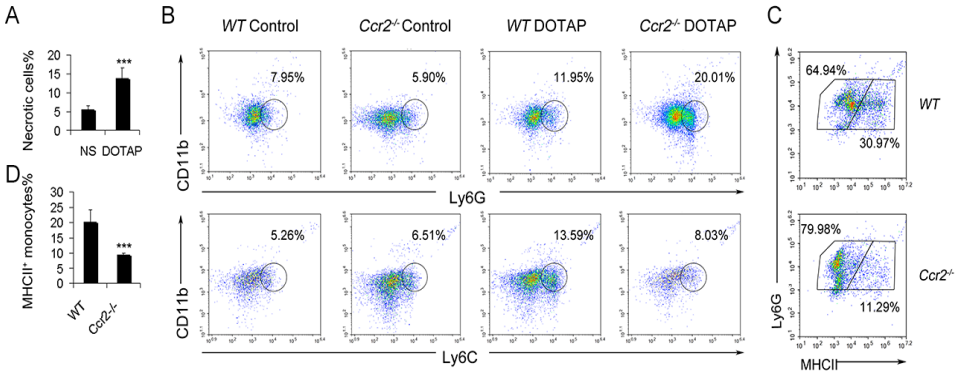
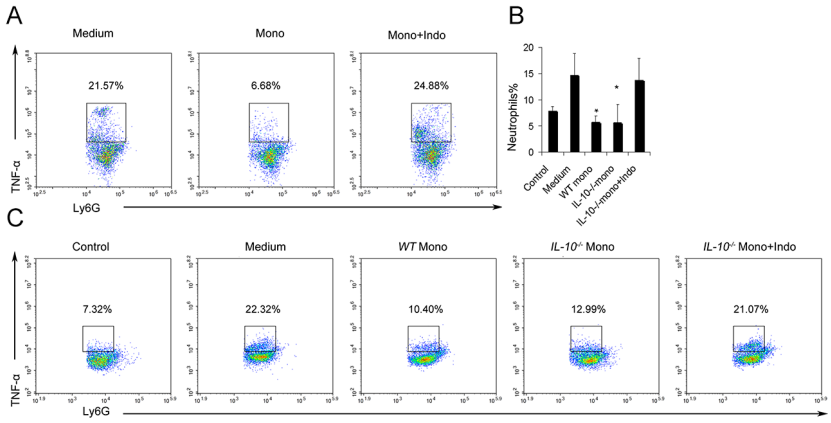


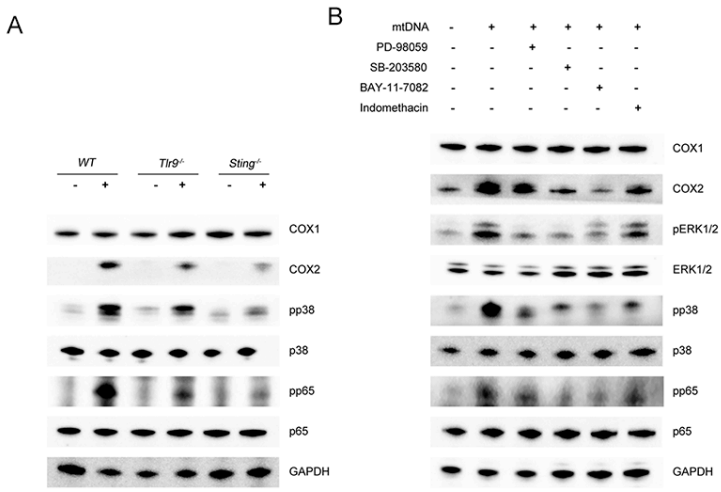
Supplementary figure 1. Pulmonary inflammation induced by the injection of cationic DOTAP liposomes. (A)C57BL/6 mice were intravenously injected with dosage of 1, 5, 10, 20, 50, 100 mg/kg cationic DOTAP liposomes. **(B)**Specific esterase staining of neutrophils in representative mouse lung sections 24 h after injection. Esterase was stained with Naphthol AS-D Chloroacetate Kit (Sigma) and procedure was performed according to the manufacturer's instruction. Influx of neutrophils in whole lungs was detected after injection with flow cytometry by staining of CD45, CD11b and Ly6G(n=3). Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).



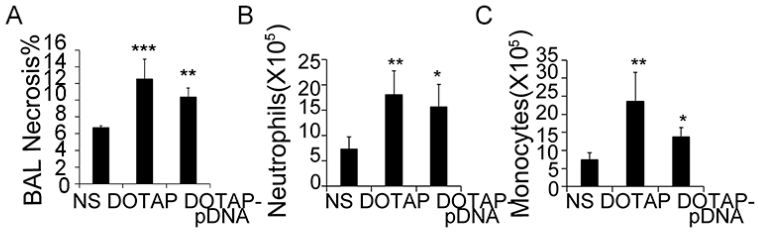
Supplementary Figure 2. Impaired Ly6C^{+} inflammatory monocytes recruitment leads to enhance inflammatory response during cationic liposomes administration. (A) C57BL/6 or *Ccr2*$^{-/-}$ mice were treated with DOTAP liposomes and sacrificed on 48 h after injection. Influx percentages of neutrophils and monocytes in lung of C57BL/6 and *Ccr2*$^{-/-}$ mice treated with DOTAP liposomes. **(B)** Assessment of ratios of neutrophils to Ly6C^{+} inflammatory monocytes in lung by flow cytometry. Cells were gated on CD11b^{+}Ly6C^{+} cells, populations were identified by staining Ly6G and MHCII. Numbers represent the percentage of cells in each gate, n=3. **(C)** MHCII^{+} monocytes percentages of C57BL/6 or *Ccr2*$^{-/-}$ mice in lung, n=3. Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).



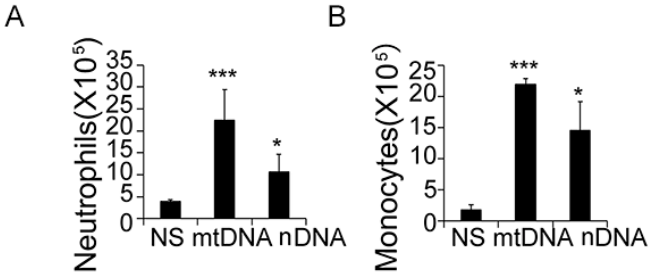
Supplementary Figure 3. *IL-10*$^{-/-}$ monocytes regulate pulmonary inflammation induced by cationic liposomes through PGE_2 production. (A) TNF-α production by neutrophils stimulated by necrotic lung cells and cultured with monocytes or in the presence of indomethacin (indo). n=3. **(B)** *WT* and *IL-10*$^{-/-}$ monocytes were isolated from C57BL/6 and *IL10*$^{-/-}$ mice bone marrow, neutrophils were stimulated with mtDNA and co-cultured with *WT* monocytes, *IL10*$^{-/-}$ monocytes and *IL10*$^{-/-}$ monocytes treated with indomethacin, or cultured medium. TNF-α production by neutrophils ($CD45^+CD11b^+Ly6G^+$) in the lung of the mice was analyzed by flow cytometry. n=3. **(C)** After stimulated with mtDNA for 20 h, *WT* monocytes, *IL-10*$^{-/-}$ monocytes and *IL-10*$^{-/-}$ monocytes treated with indomethacin were injected into C57BL/6 mice after DOTAP liposomes administration. Mice were sacrificed 48 h after administration, the monocytes ($CD45^+CD11b^+Ly6C^+$) in lung and TNF-α produced by neutrophils were determined by flow cytometry, n=3. Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).



Supplementary Figure 4. Mitochondrial DNA stimulates monocyte produce PGE_2 via TLR9- MAKP- NF-κB-COX2 pathway and also depends on STING pathway. (A) Monocytes were isolated for bone marrow of *WT, Tlr9*$^{-/-}$ and *Sting*$^{-/-}$mice and cultured with mitochondrial DNA (5 μg/mL) for 2 h at a concentration of 5×10^6 cell/mL. Western blot analysis was performed. **(B)** The freshly isolated monocytes were cultured with mitochondrial DNA (5 μg/mL) at a concentration of 5×10^6 cell/mL. Inhibitors PD9805 (30 μM), SB203580 (10 μM), BAY117082 (10 μM) and indomethacin (10 μM), were added to the cells and incubated for 2 h at 37 °C and Western blot analysis was performed. Data are representative of three independent experiments.

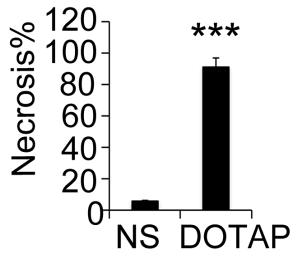


Supplementary Figure 5. (A) C57BL/6 mice were treated with intravenous injection of DOTAP liposomes (25 mg/kg), DOTAP/pVAX plasmid lipoplexes (w/w 10/1) or normal saline as control. Flow cytometry analysis of recruited cells in lung after DOTAP liposomes dosed for 24 h. Mice were killed and bronchoalveolar lavage fluid (BAL) was performed. **(B, C)** Necrotic cells in bronchoalveolar lavage fluid (BAL) were detected. Numbers indicate either $CD45^+CD11b^+Ly6C^+$monocytes or $CD45^+CD11b^+Ly6G^+$neutrophils in the quadrant expressed as number of the cells in the lung after perfusion. (n=5). Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).

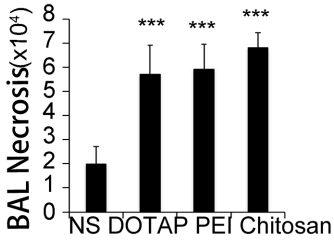


Supplementary Figure 6. (A, B) Infiltrated inflammatory neutrophils and monocytes

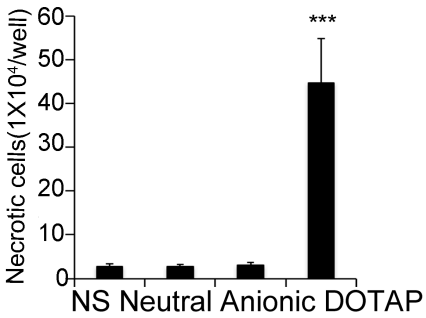
after injection of mitochondrial DNA (5 μg/mouse) and nuclear DNA (5 μg/mouse) (n=5). Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).



Supplementary Figure 7 The average of the percentages of necrotic cells induced by DOTAP liposomes (50 μg/mL). The cells were incubated with DOTAP liposomes (50 μg/mL) in RPMI 1640 supplemented with penicillin, streptomycin, HEPES and glutamine but without fetal bovine serum (FBS) in 37°C for 2 h. (n=3). Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).

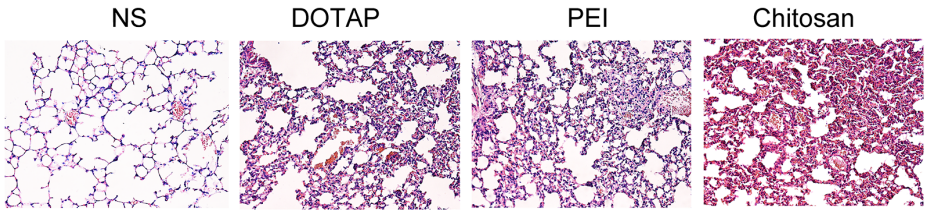


Supplementary Figure 8. C57BL/6 mice were treated with intravenous injection of liposomes, or normal saline as control. After 24 h mice were sacrificed and bronchoalveolar lavage fluid (BAL) was performed. Necrotic cells in bronchoalveolar lavage fluid (BAL) were detected. Bar graph summarizing the average numbers of necrotic cells induced by DOTAP liposomes (25 mg/kg), PEI (5 mg/kg) and chitosan (25 mg/kg) in the lung (n=5). Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (**P*<0.05; ***P*<0.01; ****P*<0.005).

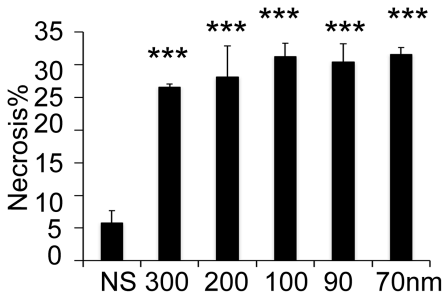


Supplementary Figure 9. Lung primary cells($1X10^6$ /well) were incubated with DOTAP liposome (20 μg/mL), Neutral liposomes (50 μg/mL) or Anionic liposomes (50 μg/mL) for 2 h and necrotic cells were detected. (n=4). Data are representative of

three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).

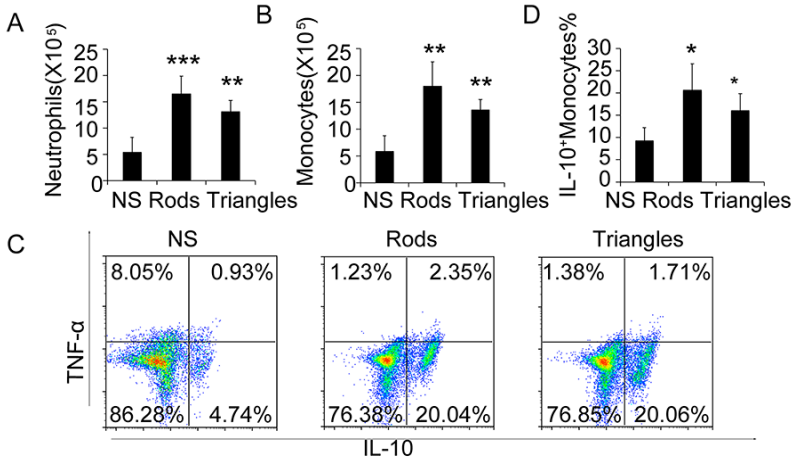


Supplementary Figure 10. Representative H&E stained section of lung from the mice treated with DOTAP liposomes (25 mg/kg), PEI (5 mg/kg) and chitosan (25 mg/kg) after 24 h (n=5), scale bars=50μm. Data are representative of three independent experiments.

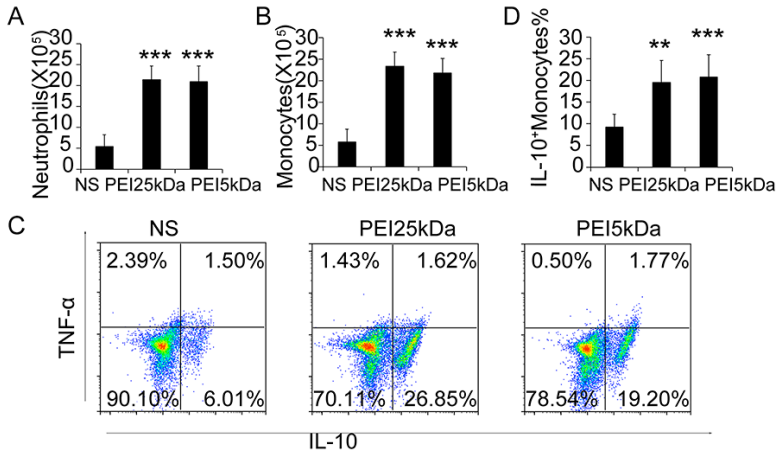


Supplementary Figure 11. Lung primary cells were incubated with DOTAP liposome (20 μg/mL) at different nanoparticle sizes (from 300 nm to 70 nm) for 2 h and necrotic cells were detected. (n=3). Data are representative of three independent

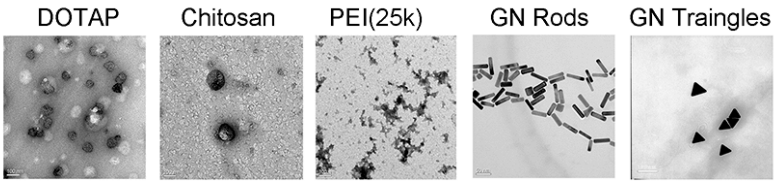
experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).



Supplementary Figure 12. Different forms of gold nanoparticles were injected to mice. Gold Nanorod (Rods) 200 μg/mouse and Gold Nanotriangle(Triangle) 200 μg/mouse. After 48 h, infiltrated inflammatory neutrophils and monocytes and the characteristics of inflammatory monocytes were detected (n=5). Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).



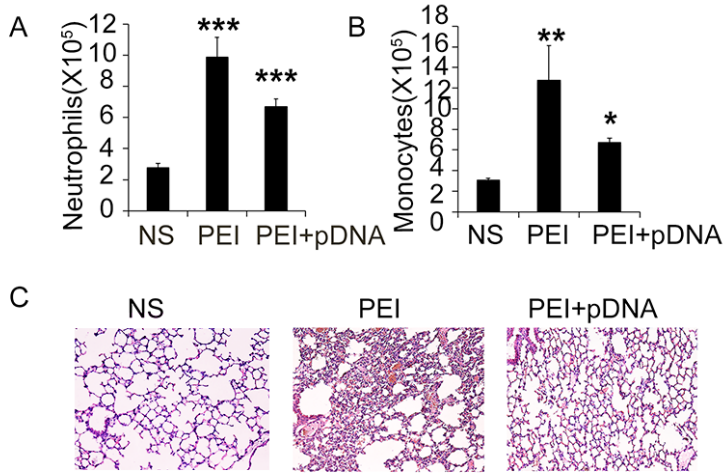
Supplementary Figure 13. Different forms of PEI were injected to mice. PEI 25kDa (5 mg/kg) and PEI 5kDa (5 mg/kg). After 48 hours, infiltrated inflammatory neutrophils and monocytes and the characteristics of inflammatory monocytes were detected (n=5). Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (**P*<0.05; ***P*<0.01; ****P*<0.005).



Supplementary Figure 14. Transmission electron microscopy (TEM) images of different types of nanoparticles. The morphologic characteristics of the nanoparticles were examined with a Tecnai G^2 F20 transmission electron microscope (TEM, FEI Company, Hillsboro, OR, U.S.).

	DOTAP	Chitosan	PEI(25k)	GN Rods	GN Traingles
Size(nm)	100.2±5.1	116.2±7.9	177.2±20.1	70.5±2.1	84.3±4.5
PDI	0.226±0.07	0.216±0.09	0.352±0.10	0.107±0.09	0.189±0.07
Charge(mV)	33.1±5.71	28.6±2.98	32.6±7.14	25.9±0.87	34.5±0.14

Supplementary Figure 15. The characteristics of different nanoparticles. The size distribution and zeta potential of the prepared liposomes were determined by Malvern Nano-ZS 90 laser particle size analyser.



Supplementary Figure 16. C57BL/6 mice were treated with intravenous injection of PEI nanoparticles (5 mg/kg), PEI/pVAX plasmid lipoplexes (w/w 5/5) or normal saline as control. Flow cytometry analysis of recruited cells in lung after administration for 24 h. Numbers indicate either $CD45^+CD11b^+Ly6C^+$ monocytes or $CD45^+CD11b^+Ly6G^+$ neutrophils in the quadrant expressed as number of the cells in

the lung after perfusion. (n=5). And Representative H&E stained section of lung from the mice treated with PEI nanoparticles (5 mg/kg), PEI/pVAX plasmid lipoplexes (w/w 5/5) or normal saline as control. Data are representative of three independent experiments, and the results are expressed as the means ± S.E.M. Statistical comparisons were performed using Student's t-test or Dunnet's t-test (*P<0.05; **P<0.01; ***P<0.005).