Electronic Supplementary Information

Cationic phosphorus dendron nanomicelles deliver microRNA mimics and microRNA inhibitors for enhanced anti-inflammatory therapy of acute lung injury

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Keywords: phosphorus dendrons; nanomicelles; alveolar macrophages; gene therapy; acute lung injury

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Additional Experimental Details

Materials. All chemicals were purchased from Aldrich, Acros, or Fluka. All solvents were freshly dried and distilled according to routine procedures before use. Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (St. Louis, MO). MH-S cells (mouse alveolar macrophages line), Roswell Park Memorial Institute 1640 (RPMI 1640) medium and fetal bovine serum (FBS) were supplied from Zhong Qiao Xin Zhou Biotechnology Co., Ltd. (Shanghai, China). Penicillin and streptomycin were from Gino Biomedical Technology Co., Ltd. (Hangzhou, China). Cell Counting Kit-8 (CCK-8), Servicebio®RT First Strand cDNA Synthesis Kit, RNA Extraction Liquid Molecular Biology Reagent, 2×SYBR Green qPCR Master Mix (High ROX), Bradford protein assay kit, Western blot kit, HRP-labeled goat anti-mouse IgG (H+L), and 4',6-diamidino-2-phenylindole (DAPI) were from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Enzyme Linked Immunosorbent Assay kits (TNF-α, IL-1β, and IL-6) were from Servicebio (Wuhan, China). The primers (Table S1) of macrophage polarization factors (TRAF6 and DUSP1) and reference gene (GAPDH) were from Generay (Shanghai, China). Enhanced green fluorescent protein plasmid DNA (EGFP-pDNA), miRNA-146a (miR-146a) mimic with and without Cy3/Cy5 labeling, miR-146a mimic scramble, miR-429 inhibitor (miR-429i) scramble, miR-429i with and without Cy3/Cy5 labeling (Table S2) were supplied by Shanghai Gene Pharma (Shanghai, China). 4S Green plus nucleic acid stain was from Sangon Biotech Co., Ltd. (Shanghai, China). Water used in all experiments was purified using a PURIST UV Ultrapure Water System (RephiLe Bioscience, Ltd., Shanghai, China) with a resistivity higher than 18.2 MΩ·cm.

NMR Characterization. ^{1}H, ^{31}P and ^{13}C NMR spectra were recorded with Bruker AV400 NMR spectrometer (Fällanden, Switzerland). All ^{31}P NMR spectra were generally recorded by avoiding the disturbance of $\{^{1}H\}$. The signal of the non-deuterated solvent served as internal standard.

Materials Synthesis (Fig. S1). *Compound 1:* 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl) (0.03 mmol) was dissolved in 2 mL dichloromethane (DCM), and

dropwise added into a 5-hydroxyisophthalicacid solution (0.01 mol, 20 mL DCM) under vigorous magnetic stirring at room temperature for 1 h. The solution of 1-dodecylamine (0.02 mol, 25 mL MeOH) was added into the 5-hydroxyisophthalicacid solution. The reaction mixture was stirred for 24 h at room temperature and then concentrated under reduced pressure. The residue was then purified by silica column chromatography (MeOH/DCM, 1/19, v/v) to afford compound 1 as a colorless oil in an 85.0% yield. **^{1}H NMR** δ= 0.90 (t, $^{3}J_{(H-H)}$ =8 Hz, 6 H, C^{g}_{0}-H), 1.28 (m, 36 H, C^{d}_{0}-H, C^{e}_{0}-H and C^{f}_{0}-H), 1.63 (m, 4 H, C^{c}_{0}-H), 3.46 (m, 4 H, C^{b}_{0}-H), 5.32 (DCM), 6.43 (t, $^{3}J_{(H-H)}$ =8 Hz, 2 H, NH-C^{a}_{0}), 7.59 (s, 2 H, C^{2}_{0}-H), 7.65 (s, 1 H, C^{4}_{0}-H) ppm. **^{13}C NMR (100 MHz, $CDCl_3$)** δ= 14.11 (s, C^{g}_{0}), 22.68 (s, C^{f}_{0}), 27.00 (s, C^{c}_{0}), 29.48 (m, C^{d}_{0}), 31.91 (s, C^{e}_{0}), 40.38 (s, C^{b}_{0}), 116.39 (s, C^{4}_{0}), 117.39 (s, C^{2}_{0}), 136.16 (s, C^{3}_{0}), 157.43 (s, C^{2}_{0}), 166.89 (s, C^{a}_{0}) ppm.

Compound 2: This compound was prepared and characterized according to the literature.[1] The solution of p-Hydroxybenzaldehyde (1.65 mol, 10 mL THF) was added into the mixture of potassium carbonate (0.18 mol) and hexachlorocyclotriphosphazene (0.30 mmol) in THF (20 mL) at 0 ℃. The reaction mixture was stirred for 24 h at room temperature and then centrifuged. The supernatant was then concentrated under reduced pressure. Crude product was purified by silica column chromatography (hexane/ethyl acetate, 3/1, v/v) to afford compound 2 as a white powder in an 87.1% yield.

Compound 3: The solution of compound 1 (1 mmol, 10 mL THF) was added into the mixture of potassium carbonate (1.9 mmol) and compound 2 (0.6 mmol) in THF (10 mL) at 0 ℃. The reaction mixture was stirred for 24 h at room temperature and then centrifuged. The supernatant was then concentrated under reduced pressure. Crude product was purified by silica column chromatography (hexane/ethyl acetate, 3/2, v/v) to afford compounds 3 as a white powder in an 80% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ= 0.89 (t, $^{3}J_{(H-H)}$ =6 Hz ,6 H, C^{g}_{0}-H), 1.27 (m, 36 H, C^{d}_{0}-H, C^{e}_{0}-H and C^{f}_{0}-H), 1.62 (m, 4 H, C^{c}_{0}-H), 3.43 (m, 4 H, C^{b}_{0}-H), 6.33 (t, $^{3}J_{(H-H)}$ =8 Hz, 2 H, NH-C^{a}_{0}), 7.17 (m, 10 H, C^{2}_{0}-H), 7.71 (m, 13 H, C^{2}_{0}-H, C^{4}_{0}-H and C^{3}_{0}-H 7, 6, 11), 9.96 (m, 5H, CHO) ppm. **^{31}P NMR (162 MHz, $CDCl_3$)** δ= 7.29 (m, P_0) ppm. **^{13}C NMR (100 MHz, $CDCl_3$)** δ=14.10 (s, C^{g}_{0}), 22.67(s, C^{f}_{0}), 27.02 (s,

$C^{c}_{\cdot 0}$), 29.49 (m, $C^{d}_{\cdot 0}$), 31.89 (s, $C^{e}_{\cdot 0}$), 40.47 (s, $C^{b}_{\cdot 0}$), 121.22 (br s, C^{2}_{0}), 121.84 (s, $C^{4}_{\cdot 0}$), 122.05 (s, $C^{2}_{\cdot 0}$), 131.37 (s, C^{3}_{0}), 133.71 (m, C^{4}_{0}), 136.86 (s, $C^{3}_{\cdot 0}$), 150.30 (m, $C^{1}_{\cdot 0}$), 154.48 (br s, C^{1}_{0}), 165.17 (s, $C^{a}_{\cdot 0}$), 190.38 (s, CHO), 190.96 (s, CHO) ppm.

Compound 5: The freshly prepared solution of compound 4 (1 mmol, 10 mL $CDCl_3$) synthesized according to the literature[2] was added into the mixture of compound 3 (0.20 mmol) and anhydrous sodium sulfate (12 mmol) in DCM (10 mL) at 0 ℃. The reaction mixture was stirred for 6 h at room temperature. Salts were removed by filtration and then the clear solution was concentrated under reduced pressure. The residue dissolved in 5 mL THF was added into 50 mL pentane and stirred for 1 h. The resulting precipitate was filtered off and dried under reduced pressure to afford compounds 5 as a white powder in a 91.5% yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ= 0.89 (t, $^{3}J_{(H\text{-}H)}$ =6 Hz ,6 H, $C^{g}_{\cdot 0}$-H), 1.26 (m, 36 H, $C^{d}_{\cdot 0}$-H, $C^{e}_{\cdot 0}$-H and $C^{f}_{\cdot 0}$-H), 1.50 (m, 4 H, $C^{c}_{\cdot 0}$-H), 3.34 (m, 4 H, $C^{b}_{\cdot 0}$-H), 3.51 (m, 15 H, CH_3-N-P_1), 6.13 (t, $^{3}J_{(H\text{-}H)}$ =4 Hz, 2 H, NH-P_1), 7.03 (m, 10 H, C^{2}_{0}-H), 7.64 (m, 17 H, $C^{2}_{\cdot 0}$-H, C^{3}_{0}-H and CH=N), 7.97 (s, 1 H, $C^{4}_{\cdot 0}$), ppm. **^{31}P NMR (162 MHz, $CDCl_3$)** δ= 8.29 (m, P_0), 62.30, 62.41 (s, P_1) ppm. **^{13}C NMR (100 MHz, $CDCl_3$)** δ= 14.13(s, $C^{g}_{\cdot 0}$), 22.68(s, $C^{f}_{\cdot 0}$), 26.99 (s, $C^{c}_{\cdot 0}$), 29.51 (m, $C^{d}_{\cdot 0}$), 31.87 (s, $C^{e}_{\cdot 0}$), 31.96 (m, CH_3-N-P_1), 40.40 (s, $C^{b}_{\cdot 0}$), 121.26 (br s, C^{2}_{0}), 121.99 (s, $C^{4}_{\cdot 0}$), 122.11 (s, $C^{2}_{\cdot 0}$), 128.62 (s, C^{3}_{0}),128.69 (s, C^{3}_{0}), 131.39 (s, C^{4}_{0}), 131.47 (s, C^{4}_{0}), 136.78 (s, $C^{3}_{\cdot 0}$), 140.60 (m, CH=N), 150.65 (s, $C^{1}_{\cdot 0}$), 151.55 (br s, C^{1}_{0}), 165.20 (s, $C^{a}_{\cdot 0}$) ppm.

Compound 6: To a solution of compound 5 (2.26 mmol) in THF (40 mL) was added 1-(2-aminoethyl) pyrrolidine (24.86 mmol) and N,N-diisopropylethylamine (27.12 mmol) at 0 ℃. The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue product dissolved in 5 mL THF was added to 100 mL pentane and the resulting precipitate was filtered off and dried under reduced pressure to afford compound 6 as a white powder in a 95.0% yield. **^{1}H NMR(400 MHz, $CDCl_3$) (400 MHz, $CDCl_3$)** δ=0.89 (t, $^{3}J_{(H\text{-}H)}$ =8 Hz, 6 H, $C^{g}_{\cdot 0}$-H), 1.26 (m, 36 H, $C^{d}_{\cdot 0}$-H, $C^{e}_{\cdot 0}$-H and $C^{f}_{\cdot 0}$-H),1.55 (m, 4 H, $C^{c}_{\cdot 0}$-H), 1.73 (br s, 40 H, C^{4}_{1}-H), 2.51 (br s, 40 H, C^{3}_{1}-H), 2.62 (m, 20 H, C^{2}_{1}-H), 3.05 (m, 20 H, C^{1}_{1}-H), 3.18 (m, 15 H, CH_3-N-P_1), 3.34 (m, 4 H, $C^{b}_{\cdot 0}$-H), 4.13 (m, 10 H, NH-P_1), 6.99 (m, 10 H, C^{2}_{0}-H), 7.53 (m, 17 H, $C^{2}_{\cdot 0}$-H, C^{3}_{0}-H and CH=N),

8.14 (s, 1 H, $C^4_{\cdot 0}$-H) ppm. **^{31}P NMR (162 MHz, $CDCl_3$)** δ= 8.39 (m, P_0), 68.52 (m, P_1) ppm. **^{13}C NMR (100 MHz, $CDCl_3$)** δ= 14.13(s, $C^g_{\cdot 0}$), 22.68(s, $C^f_{\cdot 0}$), 23.54 (s, C^4_1), 27.14 (s, $C^c_{\cdot 0}$), 29.54 (m, $C^d_{\cdot 0}$), 30.83(m, CH_3-N-P_1), 31.91 (s, $C^e_{\cdot 0}$), 39.78 (s, C^2_1), 39.90 (s, C^2_1), 40.39 (s, $C^b_{\cdot 0}$), 53.79 (s, C^3_1), 56.23 (m, C^1_1), 121.02 (br s, CH^2_0), 121.12 (s, $C^4_{\cdot 0}$), 122.01 (s, $C^2_{\cdot 0}$), 127.49 (m, C^3_0), 132.77 (s, C^4_0), 132.98 (s, C^4_0), 135.67 (s, $C^3_{\cdot 0}$), 136.02 (m, CH=N), 150.38 (br s, C^1_0), 150.78 (s, $C^1_{\cdot 0}$), 165.07 (s, $C^a_{\cdot 0}$) ppm.

Compound 7: The solution of compound 6 (0.4 mmol, 10 mL THF) was added with anhydrous sodium sulfate (12 mmol) and stirred at 0 ℃ for 20 min. The hydrogen chloride ether solution (4 mmol) was dropwise added and the resulting mixture was stirred for 1 h, and dried under reduced pressure to afford compound 7 as a white powder in a 95.0% yield.

Critical Micelle Concentration Determination. The critical micelle concentration of C12G1 was assessed using pyrene as a fluorescence probe. A series of C12G1 solutions with concentration ranging from 3.4×10^{-6} to 6.1×10^{-4} M were prepared in water. These solutions were respectively added into a flask containing the fluorescent probe pyrene at the final pyrene concentration of 6.0×10^{-7} M. Then, the mixed solutions were treated with ultrasound for 30 min and then kept at least 2 h at room temperature to ensure the micelle formation. Finally, the emission fluorescence spectra were collected at an excitation wavelength of 333 nm and slit opening of both excitation and emission at 5 nm by a QuantMaster-40 fluorescence spectrophotometer (Protein Technologies, Inc., Tucson, AZ). The fluorescence intensity ratio of I_{373} and I_{393} as a function of logarithm C12G1 concentration was analyzed to determine the CMC value.

Polyplex Preparation. C12G1 dendrons were dissolved into water to reach a final concentration of 2 mg/mL, sonicated for 30 min, and then kept at least 2 h at room temperature to obtain the C12G1 micelle solution. Comparatively, G1 dendrimers with twelve pyrrolidinium groups[3] were dissolved into water to reach a final concentration of 2 mg/mL. Polyplexes of G1/EGFP-pDNA, C12G1/EGFP-pDNA and C12G1/miRNA-mixture (miR-mixture) were prepared according to the literature[3] using different N/P ratios (the molar ratios of quaternary ammonium groups of the C12G1 or G1 to phosphates in the pDNA or miRNA backbone). Vectors and pDNA or

miR-mixture (molar ratio of miR-146a mimic and miR-429 inhibitor was 1: 1) were respectively dissolved and diluted in water, and mixed with 1 μg pDNA or miR-mixture at different N/P ratios. The mixtures were vortexed gently and then incubated at room temperature for 20 min before characterization or transfection.

Gel Retardation Assay. To evaluate the gene compression capacity of vectors, agarose gel retardation assay was carried out. An agarose gel (1% mass concentration) containing 4S green plus nucleic acid stain (0.1 μg/mL) was prepared using Tris-acetate-EDTA buffer and allowed to solidify at room temperature. Polyplexes were prepared using EGFP-pDNA or miR-mixture (1 μg) and C12G1 or G1 at different N/P ratios, naked EGFP-pDNA or miR-mixture (1 μg) was used for comparison. Gel electrophoresis was performed at 90 V for 35 min. The retardation of the polyplexes was imaged using a gel imager (Shanghai FURI Science & Technology, Shanghai, China).

Hydrodynamic Size and Zeta-Potential Measurements. C12G1 dendrons were dissolved in water (312.3 μM), sonicated for 5 min, and then kept at least 2 h at room temperature. The formed nanomicelles were diluted to have a concentration of 31.23 μM. Dynamic light scattering and zeta-potential measurements were carried out using a Malvern Zetasizer Nano-ZS Nanoseries 3 equipped with a standard 633-nm laser (Worcestershire, UK). The surface potentials and hydrodynamic sizes of the vector/pDNA or vector/miRNA polyplexes under various N/P ratios were also determined under similar conditions. The vector/pDNA or vector/miRNA polyplexes were prepared in water at the N/P ratios of 2, 5, 10, 20 or 30, where 5 μg EGFP-pDNA or miR-mixture were used to form the G1/EGFP-pDNA, C12G1/EGFP-pDNA or C12G1/miR-mixture polyplexes to reach a final volume of 1 mL. All measurements were performed at room temperature, and three parallel measurements were performed for each sample.

Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) Observation. The G1 (0.2 mg/mL), G1/EGFP-pDNA polyplexes (N/P = 2, 1 μg pDNA), C12G1 (0.2 mg/mL) and C12G1/EGFP-pDNA polyplexes (N/P = 2, 1 μg pDNA) were respectively prepared by dropping an aqueous particle suspension onto a silicon wafer and nitrogen-dried before

measurements. AFM imaging was carried out using a Molecular Force Probe 3D (MFP-3D) analytical electron microscope (Asylum Research, Santa Barbara, CA) to observe the size and morphology of the vector and vector/pDNA polyplexes. TEM imaging was executed using the JEOL 2010 analytical electron microscope (JEOL, Tokyo, Japan) at an operating voltage of 200 kV. Particularly, C12G1 micelles (1 mg/mL) or C12G1/EGFP-pDNA polyplexes (N/P = 2, 1 μg pDNA) were prepared by dropping an aqueous solution of the material onto a carbon-coated copper grid and lamp-dried before measurements. The particle size distribution was measured using Image J 1.40 G software (http://rsb.info.nih.gov/ij/download.html). For each sample, at least 100 particles from different images were randomly selected and analyzed.

Cytotoxicity Assay. The cytocompatibility of the G1 dendrimers, G1/gene polyplexes, C12G1 micelles or C12G1/gene polyplexes (gene represents EGFP-pDNA or miR-mixture) were assessed by CCK-8 cell viability assay according to the literature.[3] In brief, MH-S cells were seeded into 96-well plates at a density of 8000 cells per well with 100 μL RPMI 1640 medium containing 10% FBS, 0.05 mM β-mercaptoethanol, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 ℃ and 5% CO_2 overnight. The next day, the cells were incubated with 2 μg/mL LPS for 24 h to be activated. Then, the medium was replaced with fresh RPMI 1640 containing G1 or C12G1 (10 μL) in PBS (phosphate buffered solution, pH = 7.4) with different final concentrations (93.5-3000 $\times 10^{-9}$ M), or G1/gene and C12G1/gene polyplexes (the dose of EGFP-pDNA or miR-mixture was 1 μg per well). The cells were then incubated at 37 ℃ for 24 h. Cells treated with PBS were used as control. After that, the medium of each well was replaced with serum-free RPMI 1640 containing CCK-8 (10% v/v) and the cells were incubated at 37 ℃ for additional 3 h. The absorbance of each well at 450 nm was measured by Thermo Scientific Multiskan MK3 enzyme linked immunosorbent assay (ELISA) reader (Thermo Scientific, Waltham, MA). For each sample, five parallel wells were analyzed.

Gene Transfection Efficiency of EGFP-pDNA. G1 dendrimers or C12G1 dendron micelles were mixed with EGFP-pDNA to form polyplexes at different N/P ratios. MH-S cells were seeded in 12-well plates at a density of 1 $\times 10^5$ cells per well and cultivated overnight under 37 ℃ and 5%

CO_2. The next day, the cells were incubated with 2 μg/mL LPS for 24 h to be activated. The medium of each well was replaced with fresh RPMI 1640 (without FBS) containing G1/EGFP-pDNA or C12G1/EGFP-pDNA polyplexes (1 μg of pDNA per well at N/P ratios of 5, 10, 20 and 30, respectively). After 4 h, the medium in each well was replaced with serum-containing RPMI 1640 and the cells were cultivated for 24 h. Then, the medium was removed and cells were washed with PBS for three times. The cells were then fixed with 2.5% glutaraldehyde for 20 min at room temperature and washed with PBS for three times. The expression of EGFP protein in activated MH-S cells was observed by Axio Vert.A1 Carl Zeiss fluorescence microscopy (Jena, Germany). Meanwhile, flow cytometric analysis was also used to quantitatively investigate the EGFP expression after transfection of the G1/EGFP-pDNA and C12G1/EGFP-pDNA polyplexes. Flow cytometry was carried out using a Becton Dickinson Facscan analyzer (Franklin Lakes, NJ). The cells were washed with PBS for three times, digested with trypsin-EDTA, resuspended in 200 μL PBS, and measured in the FL1-H channel. Each measurement was sampled with 1×10^4 cells (n = 3).

In Vitro **Cellular Uptake of C12G1/MiR-Mixture Polyplexes.** C12G1 micelles were mixed with Cy3-miR-mixture to form the polyplexes at different N/P ratios (2, 5, 10, 20 or 30). MH-S cells were seeded in 12-well plates at a density of 1×10^5 cells per dish, cultivated overnight under 37 ℃ and 5% CO_2, and activated by incubation with LPS (2 μg/mL) for 24 h. The cells were then treated with C12G1/Cy3-miR-mixture polyplexes (100 μL) containing 1 μg of Cy3-miR-mixture at an N/P ratio of 2, 5, 10, 20 or 30, and cultured with serum-containing RPMI 1640 for 4 h at 37 ℃. Afterwards, the medium was removed and cells were washed with PBS for three times. To quantitatively evaluate the cellular uptake efficiency, flow cytometry analysis was performed. The cells were suspended in 200 μL PBS and measured in the FL2-H channel with each measurement sampled with 1×10^4 cells (n = 3). To qualitatively evaluate the cellular uptake efficiency, the cells were fixed with 2.5% glutaraldehyde for 20 min at room temperature, washed with PBS for three times, and counter stained with DAPI (2 μg mL^{-1}) at room temperature for 5 min. Intracellular localization of different polyplexes in MH-S cells was observed by laser scanning confocal

microscope (ZEISS LSM-700, Jena, Germany) at 63 × oil-immersion objective lens.

Inflammatory Cytokine Expression *in Vitro*. MH-S cells were seeded into 12-well culture plates at a density of 1×10^5 cells per well with 1 mL of fresh RPMI 1640 medium at 37 ℃ and 5% CO_2 overnight, and activated with 2 μg/mL LPS for 24 h. Then, cells in each well were treated with serum-free medium containing C12G1/miR-mixture, C12G1/miR-146a mimic+scramble, C12G1/miR-429i +scramble, miR-mixture, or C12G1. After 4 h, the medium in each well was replaced with serum-containing RPMI 1640 medium and cells were cultivated for 24 h. Cells treated with PBS were used as positive control and normal cells treated with PBS were used as negative control. After cultivation for 24 h, culture medium of each well was collected to analyze the inflammatory factors (TNF-α, IL-1β, and IL-6) using commercial ELISA kits, and inflammatory mediator NO using commercial Griess Reagent kits.

Gene Therapy Evaluation *in Vitro.* MH-S cells were seeded into 6-well culture plates at a density of 2×10^5 cells per well with 1 mL of fresh RPMI 1640 medium at 37 ℃ and 5% CO_2 overnight, and activated with 2 μg/mL LPS for 24 h. Then, cells in each well were treated with serum-free medium containing C12G1/miR-mixture, C12G1/miR-146a mimic+scramble, C12G1/miR-429i+scramble, miR-mixture, or C12G1. After 4 h, the medium in each well was replaced with serum-containing RPMI 1640 medium and the cells were cultivated for 24 h. Cells treated with PBS were used as positive control and normal cells treated with PBS were used as negative control. After that, cells were collected in centrifuge tubes, washed with PBS for three times, and added with 200 μL lysis buffer in an ice-bath for 30 min. The lysates were analyzed *via* Western blot assays to detect TRAF6 and DUSP1 protein expression according to the literature protocols.[4] *β-actin* was employed as a reference protein. Meanwhile, the miR-146a mimic and miR-429i downstream regulatory mRNA (TRAF6 and DUSP1) expression levels within cells were determined using RT-PCR (Stepone plus, Applied Biosystems, Carlsbad, CA) according to the above protocols.[5] In brief, total RNA was extracted from the MH-S cells utilizing RNA Extraction Liquid Molecular Biology Reagent, and first strand cDNA was generated by Servicebio®RT First

Strand cDNA Synthesis Kit. The amplification was performed in 40 cycles by a fluorescence detection system (MJ Research) with SYBR green fluorescence. Each cycle consisted of heat denaturation for 15 s at 95 ℃, annealing, and extension for 30 s at 60 ℃. All samples were quantified using a $2^{-\Delta\Delta Ct}$ method to calculate the relative mRNA expression using the reference gene *GAPDH* (n = 3). The upstream and downstream primer sequences of each gene are shown in Table S1.

***In Vivo* ALI Therapy.** All animal experiments were performed in accordance with the guidelines of the Committee on Experimental Animal Care and Use of Donghua University and also following the regulations of the National Ministry of Health. Six-week-old Male Balb/c mice (Shanghai Slac Laboratory Animal Center, Shanghai, China) were aerosolized and inhaled with LPS solution (5 mg/kg) to induce ALI. At 24 h post LPS challenge, C12G1/miR-mixture polyplexes (N/P = 10, miR-mixture = 20 μg), C12G1/miR-146a mimic + scramble polyplexes (N/P = 10, miR-146a mimic + scramble = 20 μg), C12G1/miR-429i + scramble polyplexes (N/P = 10, miR-429i + scramble = 20 μg) and miR-mixture polyplexes (20 μg) were respectively aerosolized and inhaled by each mouse. ALI mice and normal mice treated with PBS were used as positive and negative control, respectively. After 24 h, animals were sacrificed to obtain lung tissues and bronchoalveolar lavage fluid (BALF). Lung tissues were collected and weighed to obtain the "wet" weight, dried at 80 °C for 72 h to obtain the "dry" weight, and the wet/dry weight ratio was calculated to evaluate the anti-inflammatory therapy effect of lung tissue. BALF was centrifuged (1000 rpm for 5 min) to collect the supernatant for quantification of pro-inflammatory cytokines using commercial ELISA kits. In addition, the protein expression level of polarization factors (TRAF6 and DUSP1) in lung tissues were evaluated by Western blotting according to protocols reported in the literature.[4] *β-actin* was used as reference protein.

***In Vivo* Biodistribution.** To investigate the biodistribution of the C12G1/miR-mixture polyplexes, each ALI mouse was administrated with the C12G1/Cy5-miR-mixture polyplexes according to the above protocols. The fluorescence imaging of the ALI mice at different time points

(4 and 24 h) was carried out using a commercial VISQUE *In Vivo* Smart imaging system (Vieworks Co., Ltd., Gyeonggi-do, Korea). To investigate the fluorescence signal biodistribution after different administrations, the mice were euthanized after imaging, and the heart, liver, spleen, lung and kidney were extracted for *ex vivo* fluorescence imaging. All fluorescence imaging data at the regions of interest (ROI) were acquired and analyzed using the Smart imaging software.

Histological Examinations and Micro-Computed Tomography (micro-CT) Imaging. One mouse of each group was euthanized at 24 h post treatment. Then, the heart, liver, spleen, lung and kidney were harvested and sectioned for hematoxylin-eosin (H&E) staining. Both stainings were carried out according to standard protocols.[6, 7] The inflammatory lung injury severity of each mouse was scored according to the following four items: alveolar congestion, haemorrhage, infiltration or aggregation of neutrophils in the vessel wall, and thickness of the alveolar wall, where 0 = minimal (little) damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage, and 4 = maximal damage.[6] Meanwhile, another mouse of each group was euthanized and lung tissues were harvested and fixed in paraformaldehyde (4%) at room temperature for 48 h. The mouse lung tissues were also assessed by micro-CT system (SCANCO Medical AG, Zurich, Switzerland). The CT scanning parameters were set at a voltage of 45 kV, a current of 200 μA, and an exposure time of 300 ms. The volumes of lung tissue were measured from the reconstructed three dimensional CT images.

Statistical Analysis. One-way analysis of variance statistical method was adopted to evaluate the significant difference of data between groups using IBM SPSS Statistic 26 software (IBM, Armonk, NY). A value of 0.05 was considered as the level of significance, and the associated data were marked as (*) for $p < 0.05$, (**) for $p < 0.01$, (***) for $p < 0.001$, respectively.

Table S1. The sequences of upstream, downstream, and stem loop primers.

Factors	Sense	Antisense
TRAF6	5′- GGTGTAAGGCCTAGCAGCAGAT-3′	5′- AAATAGCCCATGGAAGCACAGT-3′
DUSP1	5′- GAGCTGTGCAGCAAACAGTC-3′	5′- CTTCCGAGAAGCGTGATAGG-3′
GAPDH	5′-CCTCGTCCCGTAGACAAAATG-3′	5′-TGAGGTCAATGAAGGGGTCGT-3′

Table S2. The sequences of (1) miR-146a mimic with and without Cy3/Cy5 labeling, (2) miR-146a mimic scramble, (3) miR-429i with and without Cy3/Cy5 labeling, and (4) miR-429i scramble.

Factors	Sense	Antisense
1	5′- UGAGAACUGAAUUCCAUGGGUU-3′	5′- CCCAUGGAAUUCAGUUCUCAUU-3′
2	5′- UUCUCCGAACGUGUCACGUTT-3′	5′- ACGUGACACGUUCGGAGAATT-3′
3	5′-ACGGCAUUACCAGACAGUAUUA-3′	
4	5′- CAGUACUUUUGUGUAGUACAA-3′	

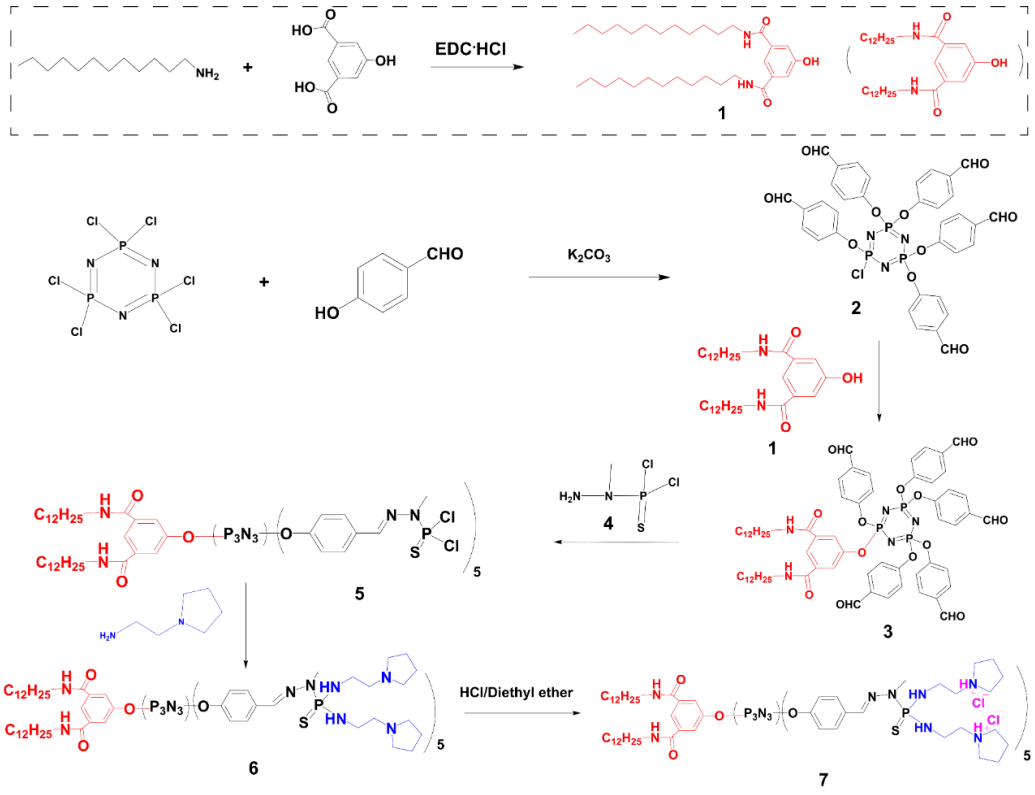

Fig. S1. Synthesis of cationic phosphorous dendron (C12G1).

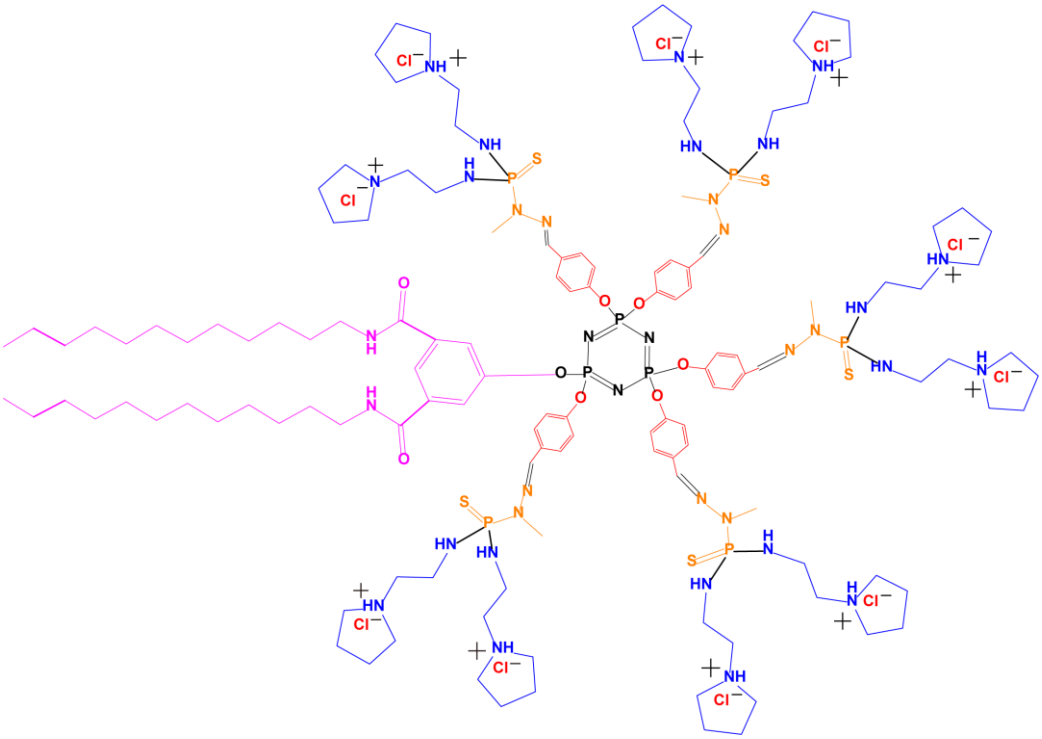

Fig. S2. 2D chemical structure of C12-G1 dendron.

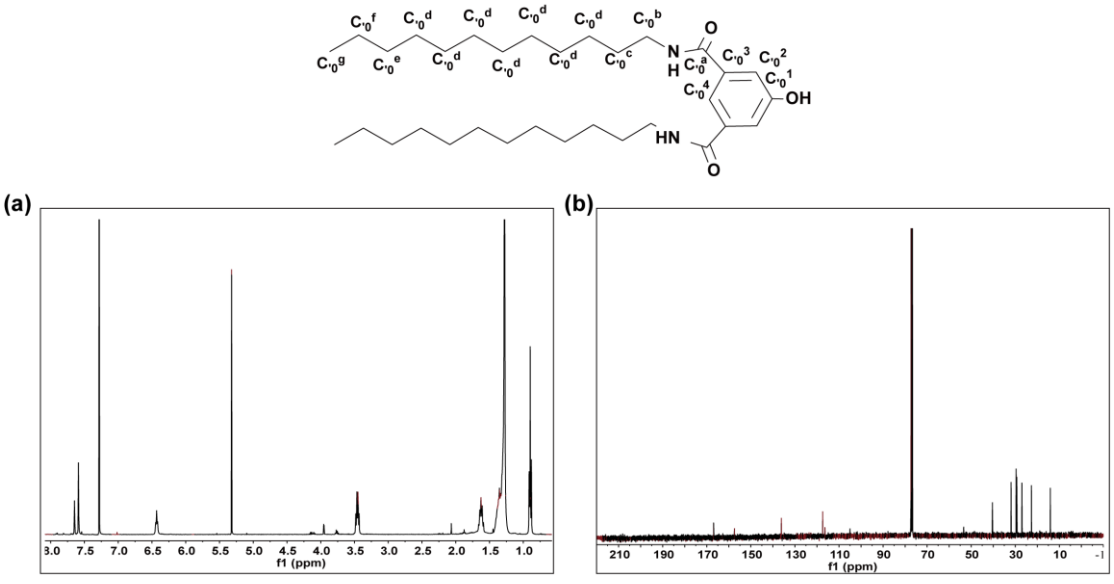

Fig. S3. ^{1}H NMR (a) and ^{31}C NMR (b) spectra of compound 1.

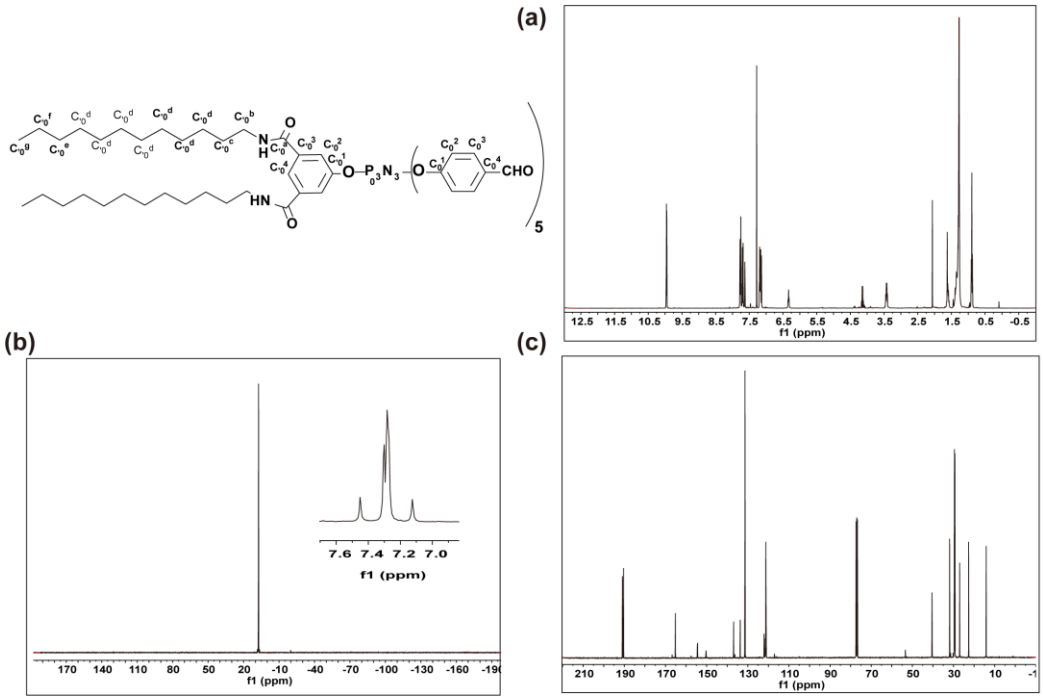

Fig. S4. ^{1}H NMR (a), ^{31}P{^{1}H NMR (b) and ^{13}C NMR (c) spectra of compound 3.

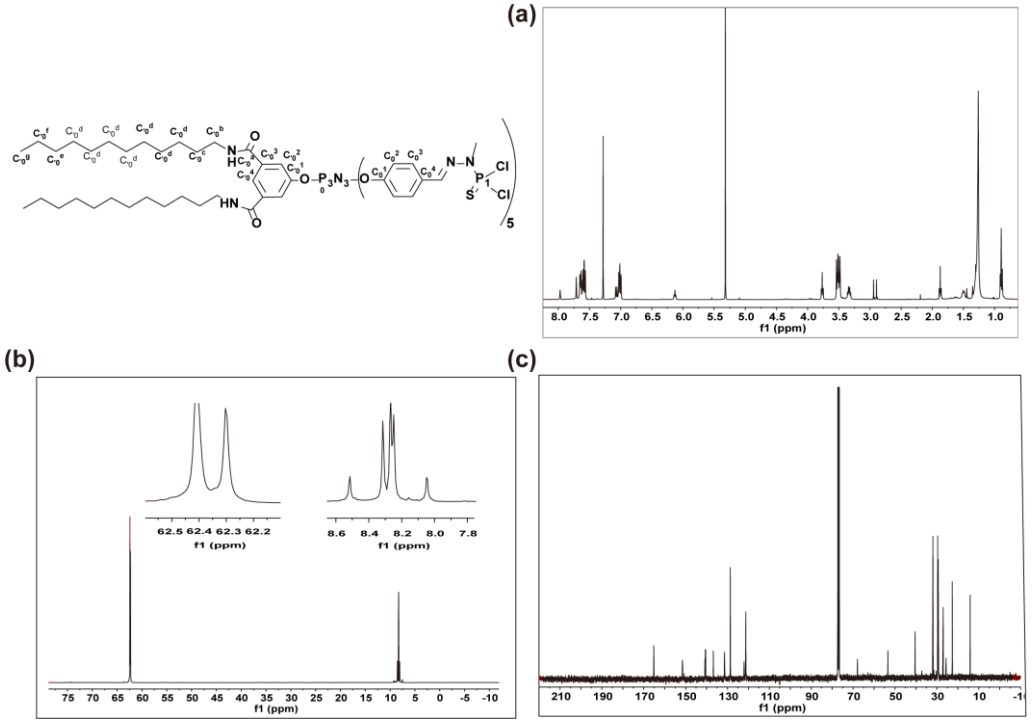

Fig. S5. ^{1}H NMR (a), ^{31}P{^{1}H} NMR (b) and ^{13}C NMR (c) spectra of compound 5.

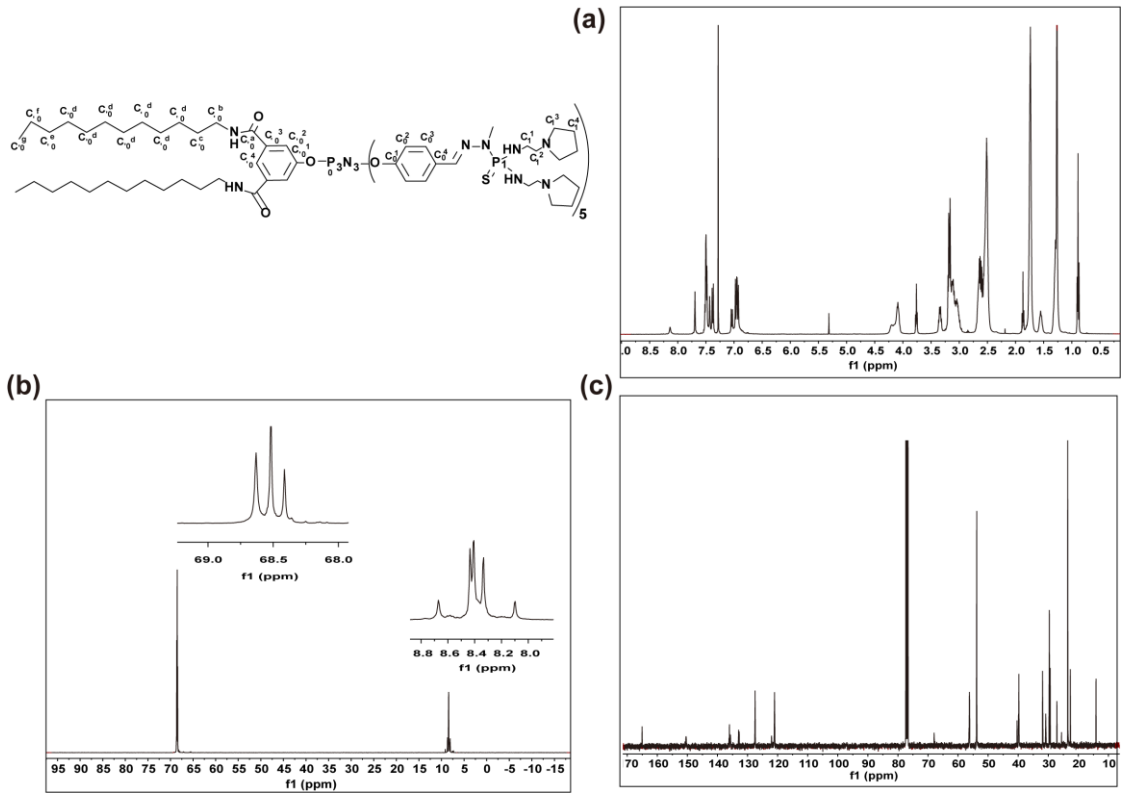

Fig. S6. ^{1}H NMR (a), $^{31}P\{^{1}H\}$ NMR (b) and ^{13}C NMR (c) spectra of compound 6.

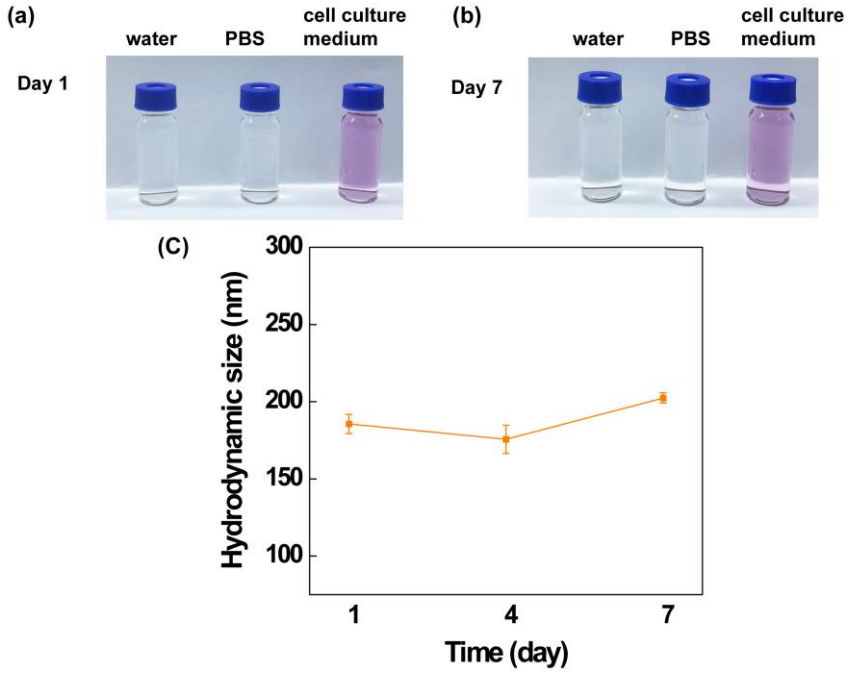

Fig. S7. Photos of the aqueous solutions of C12G1 dendron (312.7 μM) dispersed in different solvents on day 1 (a) and day 7 (b). (c) Time-dependent size evolution of C12G1 dendron micelles dispersed in water.

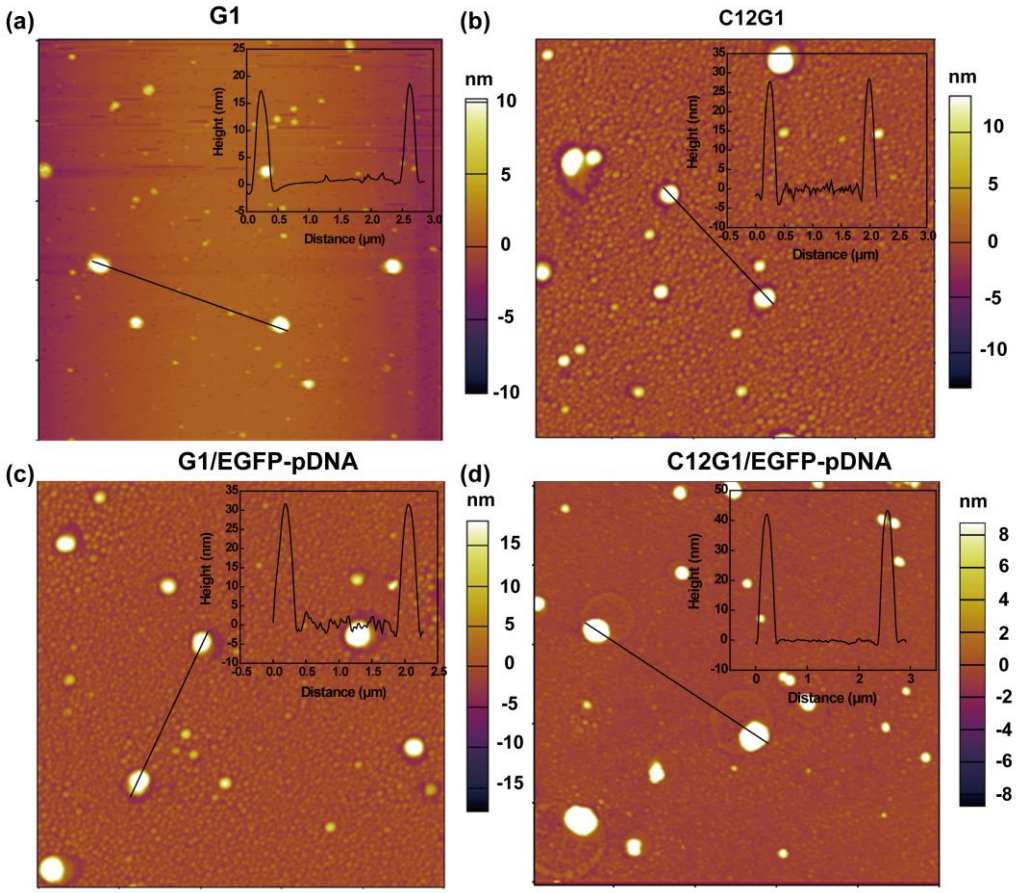

Fig. S8. AFM image and corresponding height profile of (a) G1 dendrimers, (b) C12G1 dendron micelles, (c) G1/EGFP-pDNA polyplexes and (d) C12G1/EGFP-pDNA polyplexes.

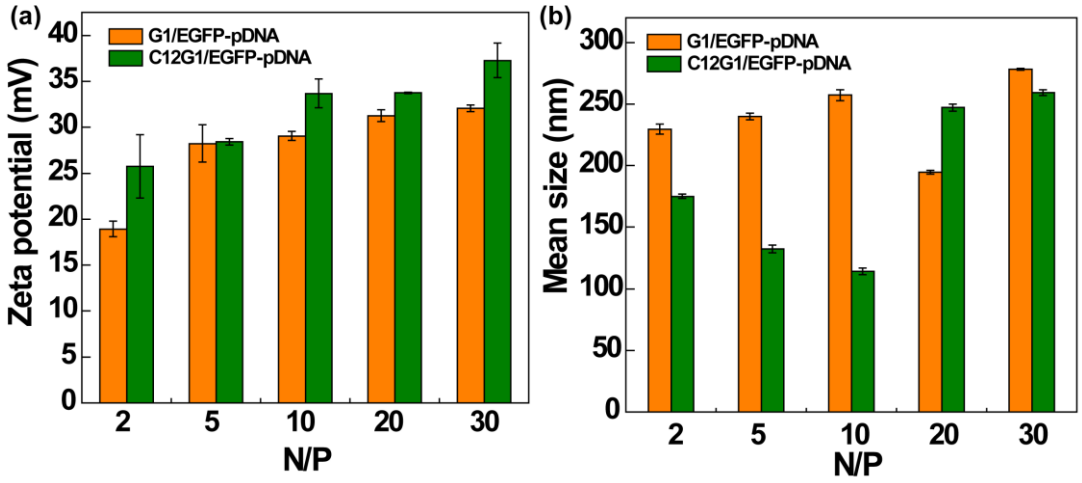

Fig. S9. (a) surface potential and (b) hydrodynamic size of G1/EGFP-pDNA and C12G1/EGFP-pDNA polyplexes under different N/P ratios (n = 3).

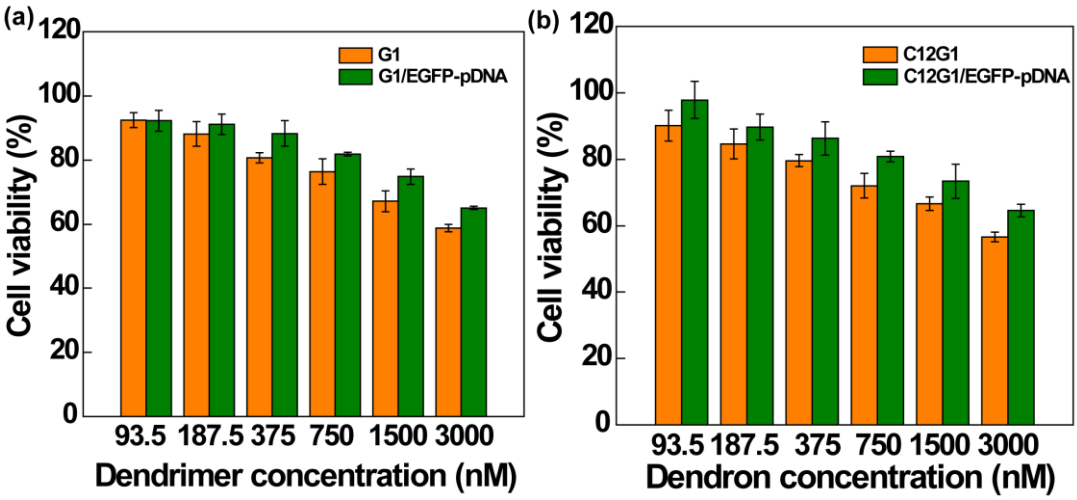

Fig. S10. CCK-8 viability assay of activated MH-S cells treated with (a) G1 and G1/EGFP-pDNA polyplexes, and (b) C12G1 and C12G1/EGFP-pDNA polyplexes (n = 5).

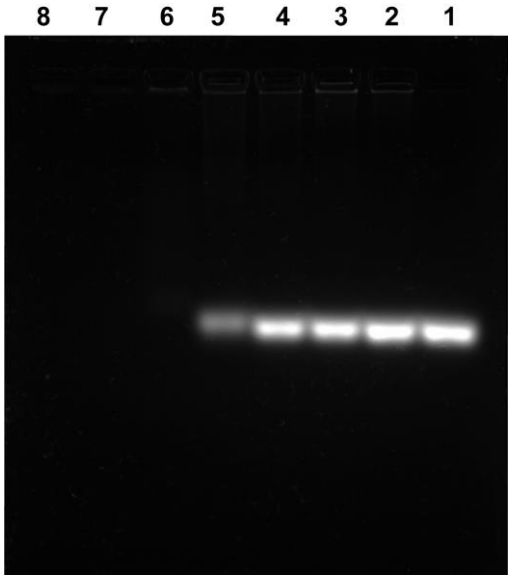

Fig. S11. Gel retardation assay of C12G1/miR-mixture polyplexes at different N/P ratios (the molar ratio of quaternary ammonium groups of the dendron to phosphates in the RNA backbone). Lane 1, naked miR-mixture; lane 2, N/P = 0.0625: 1; lane 3, N/P = 0.125: 1; lane 4, N/P = 0.25: 1; lane 5, N/P = 0.5: 1; lane 6, N/P = 1: 1; lane 7, N/P = 2: 1; and lane 8, N/P = 4: 1.

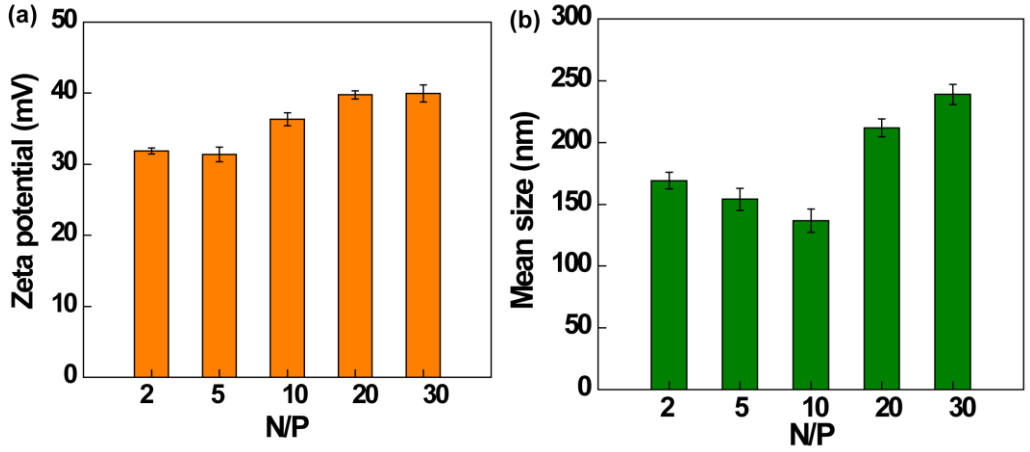

Fig. S12. (a) Surface potential and (b) hydrodynamic size of C12G1/miR-mixture polyplexes under different N/P ratios (n = 3).

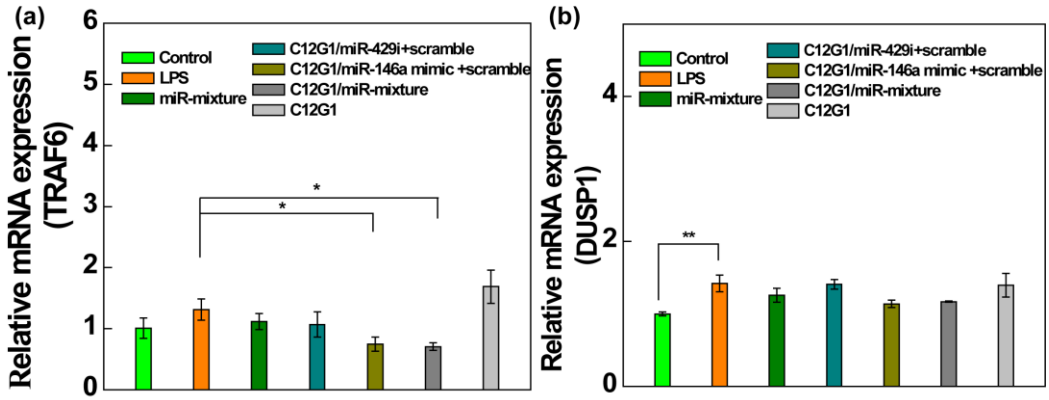

Fig. S13. RT-PCR assay of mRNA expression of (a) TRAF6 and (b) DUSP1 after the LPS-activated MH-S cells were treated with different materials (n = 3).

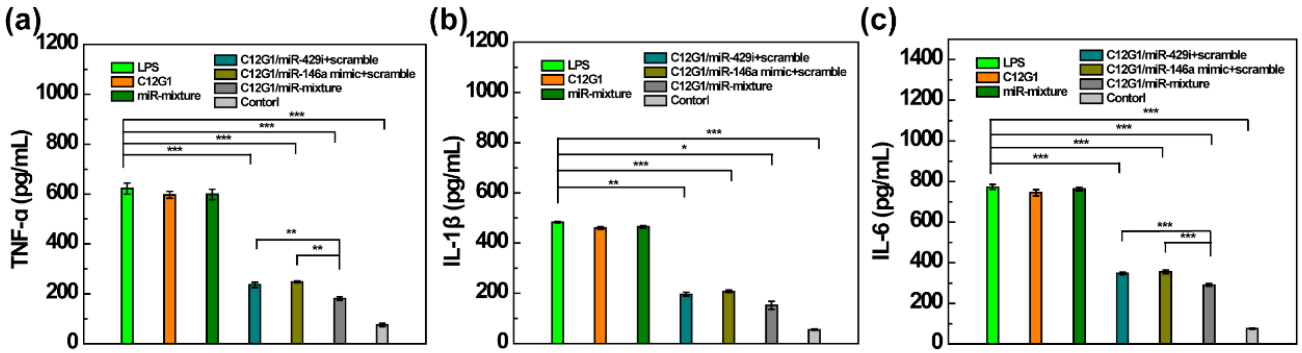

Fig. S14. ELISA of the inflammatory cytokines of (a) TNF-α, (b) IL-1β, and (c) IL-6 in the cell culture medium secreted by MH-S cells after treatment with different materials (n = 3).

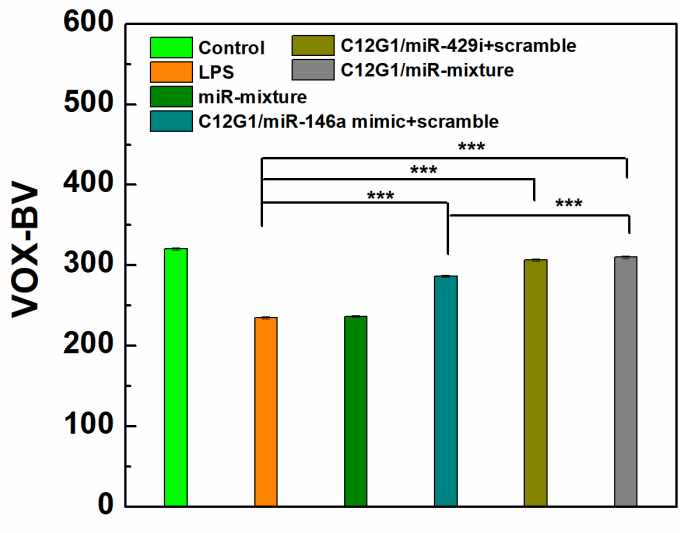

Fig. S15. The lung tissue volumes (VOX-BV) of mice at 24 h post treatment in different groups (n = 3).

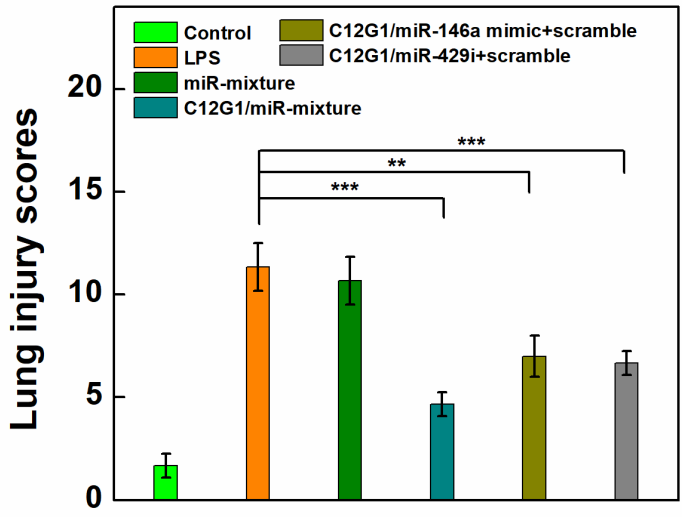

Fig. S16. The lung injury scores of mice at 24 h post treatment in different groups (n = 3).

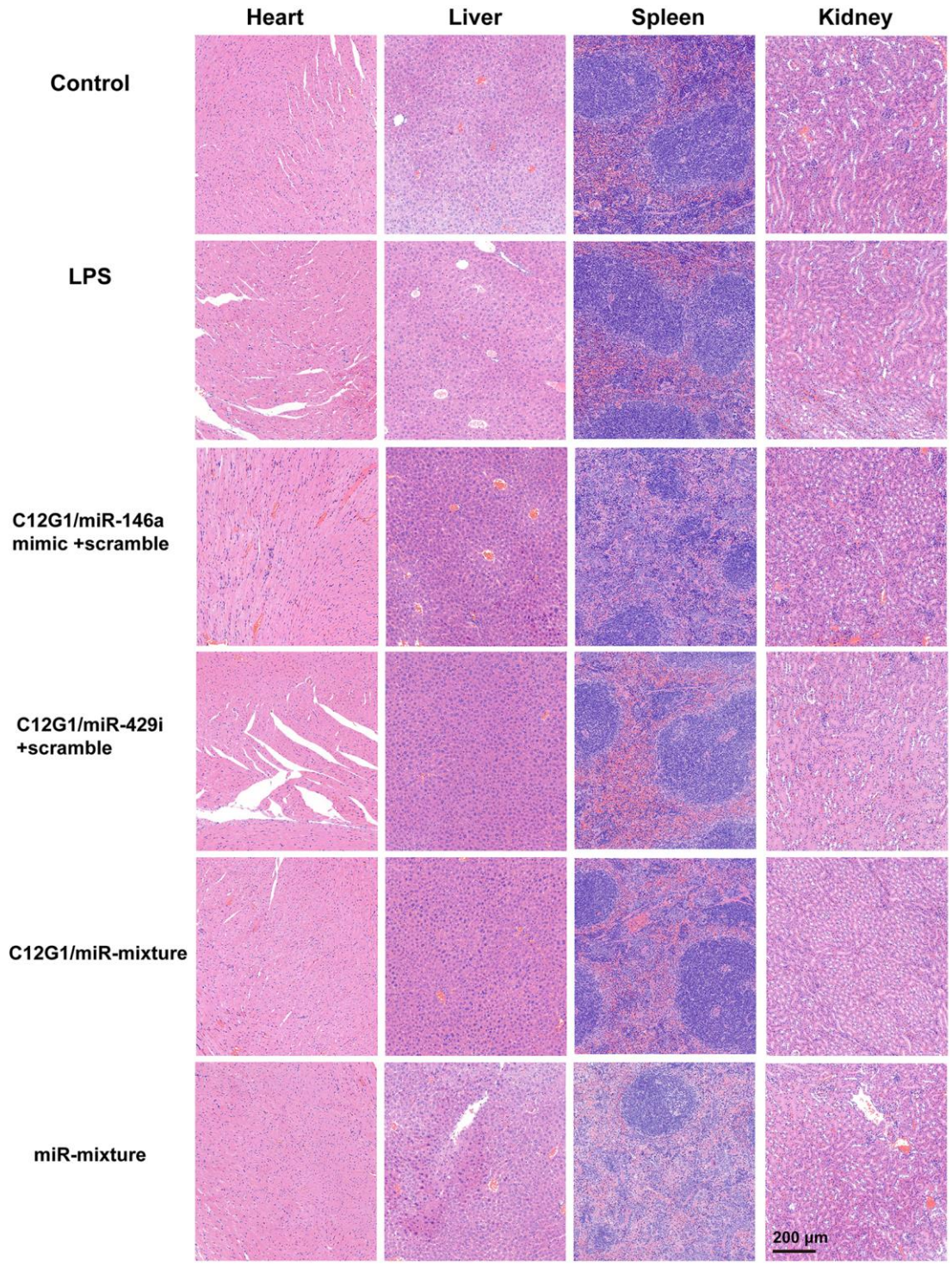

Fig. S17. H&E-stained images of major organs (heart, liver, spleen, and kidney) extracted from different groups at 24 h post treatment. Normal mice administrated with PBS were used as negative control and LPS-induced ALI mice administrated with PBS were used as positive control.

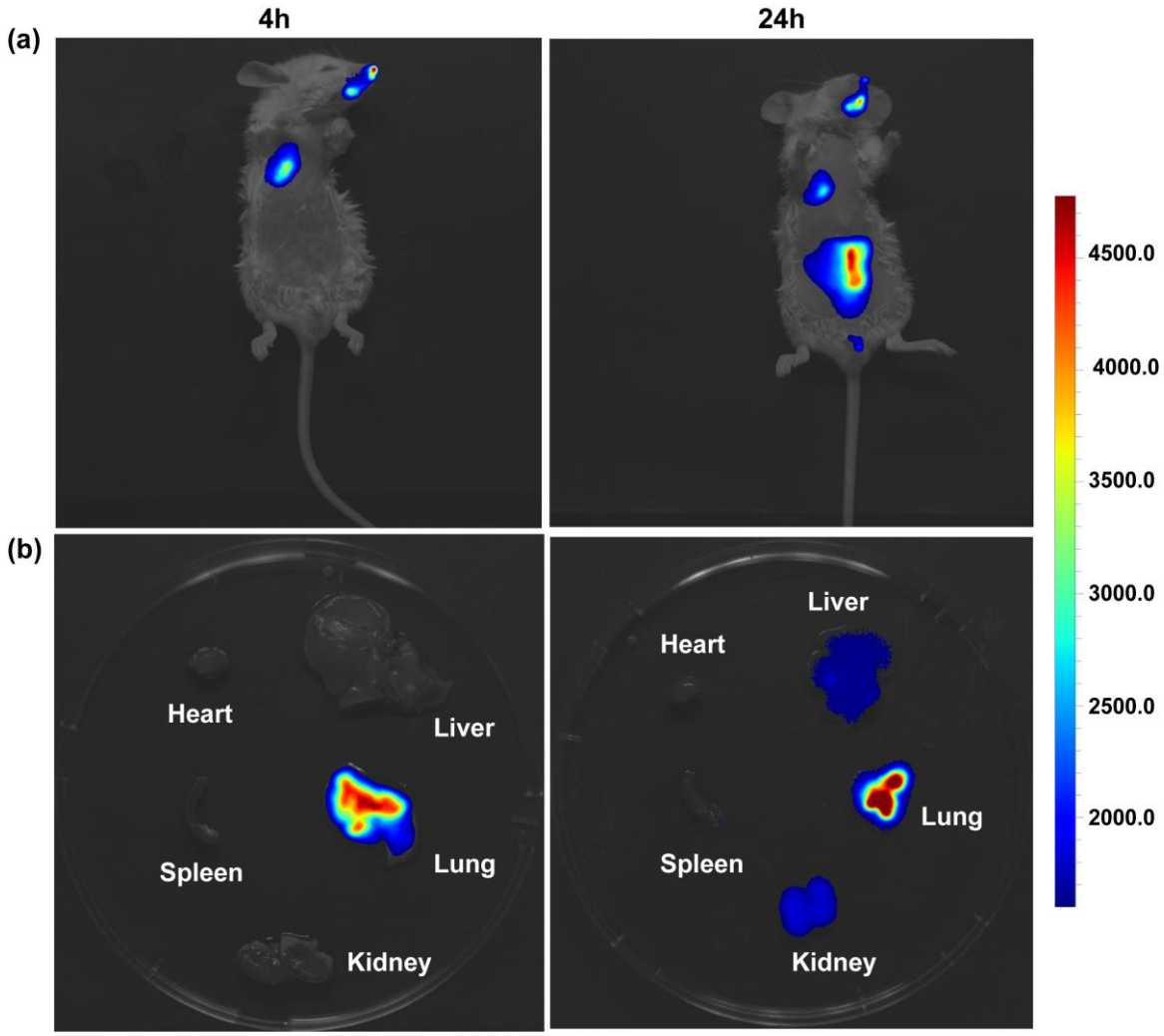

Fig. S18. (a) *In vivo* and (b) *ex vivo* fluorescence images of ALI mice and mouse organs, respectively, at 4 h and 24 h post administration of C12G1/Cy5-miR-mixture.

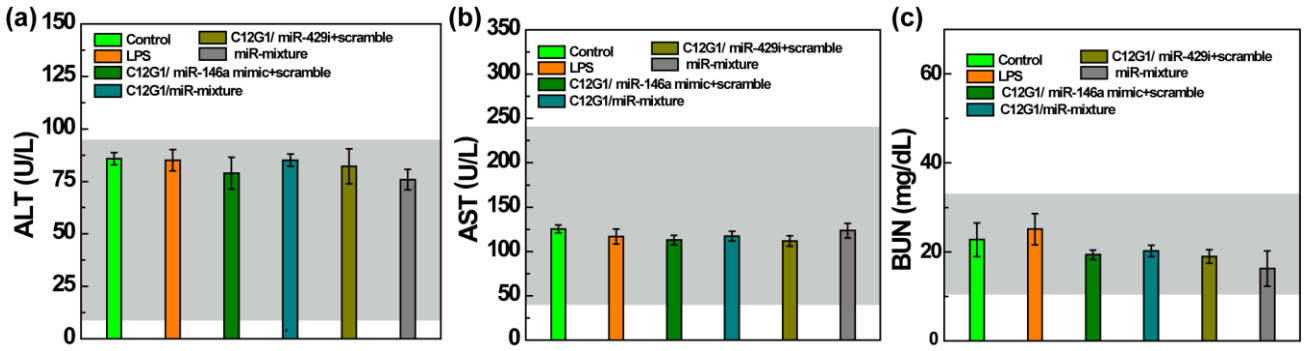

Fig. S19. *In vivo* assays of blood biochemical indices after ALI mice were intratracheally atomized with miRNA-mixture, C12G1/miRNA146a mimic + scramble, C12G1/miRNA429i + scramble, or C12G1/miRNA-mixture and sacrificed at 24 h post treatment for blood collection (n = 3). The blood biochemical data include (a-b) liver function indexes (alanine aminotransferase (ALT), and aspartate aminotransferase (AST)) and (c) kidney function index urea nitrogen (BUN). Gray areas indicate the referred normal ranges for healthy mice obtained from Servicebio, Inc. (Wuhan, China) using Hematology Analyzer (Mindray, BC-2800 vet).

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