Supporting information

Fluorinated α-Helical Polypeptides Synchronize Mucus Permeation and Cell Penetration Toward Highly Efficient Pulmonary siRNA Delivery Against Acute Lung Injury

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Materials, cell lines, and animals.

P-hydroxybenzyl alcohol, L-glutamic acid (99%), copper bromide (CuBr, 99%), *N*,*N*,*N'*,*N''*,*N''*-pentamethyldiethylenetriamine (PMDETA, 99%), butyric anhydride, and lipopolysaccharide (LPS, from Escherichia coli 0111:B4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-Bromopropyne, triphosgene, *n*-butylamine, trifluoroacetic anhydride, pentafluoropropionic anhydride, heptafluorobutyric anhydride, 4-dimethylaminopyridine (DMAP), 6-chlorohexanol, and tetramethylguanidine (TMG) were purchased from Energy Chemical (Shanghai, China). Dimethylformamide (DMF), tetrahydrofuran (THF), and hexane were dried by a column packed with alumina before use. γ-(4-Propargyloxybenzyl)-L-glutamic acid based *N*-carboxyanhydride (POBLG-NCA) and 6-azidohexyl guanidine were synthesized according to reported procedures.[1] PrimeScript RT kit and SYBR Premix Ex Taq kit were purchased from Takara (Beijing, China), and ELISA kits were purchased from Invitrogen (Carlsbad, CA). MPO kit was purchased from eBioscience (San Diego, CA, USA). TNF-α siRNA (siTNF-α), negative control siRNA (siNC), and primers were purchased from GenePharma (Shanghai, China), and their sequences were shown in Tables S1 and S2.

Table S1. Sequences of siTNF-α and siNC.

	Sequence
siTNF-α sense	5'-GUCUCAGCCUCUUCUCAUUCCUGCT-3'
siTNF-α antisense	5'-AGCAGGAAmUGmAAmGAGGmCUGAmGACmAmU-3'
siNC sense	5'-UUCUCCGAACGUGUCACGUTT-3'

siNC antisense	5’-ACGUGACACGUUCGGAGAATT-3’

Table S2. Primer sequences of TNF-α and GAPDH.

	Sequence
TNF-α F	5’-CCCTCACACTCAGATCATCTTCT-3’
TNF-α R	5’-GCTACGACGTGGGCATCAG-3’
GAPDH F	5’-TTCACCACCATGGAGAAGGC-3’
GAPDH R	5’-GGCATGGACTGTGGTCATGA-3

RAW 264.7 (mouse monocyte macrophage) and Calu-3 (human lung adenocarcinoma) cells were purchased from the American Type Culture Collection and cultured in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

Male Balb/c mice (6−8 weeks) were obtained from Shanghai Slaccas Experimental Animal Co., Ltd. and were housed in a clean room, five to a cage. All animal experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee, Soochow University.

Instrumentation.

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian ^{UNITY}INOVA-400 spectrometer at room temperature. Chemical shifts (δ) were reported in the units of ppm and referenced to the protonic impurities. Gel permeation chromatography (GPC) analyses were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN

HELEOS multi-angle laser light scattering detector (MALLS) detector (Wyatt Technology, Santa Barbara, CA, USA), and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100 Å, 500 Å, 10^3 Å and 10^4 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60 ℃ using DMF containing 0.05 M LiBr as the eluent phase at a flow rate of 1.0 mL/min. The MALLS detector was calibrated using pure toluene with no need for calibration using polymer standards and can be used for the determination of the absolute molecular weights (MWs). The MWs of polymers were determined based on the d*n*/d*c* value of each polymer sample calculated offline by using the internal calibration system processed by the ASTRA V software (version 5.1.7.3, Wyatt Technology, Santa Barbara, CA, USA). Circular dichroism (CD) measurements were carried out on a JASCO J-700 CD spectrometer. Polypeptides were dissolved in DI water at 0.2 mg/mL unless otherwise specified. The solution was placed in a quartz cell with a path length of 1 mm. The mean residue molar ellipticity of each polypeptide was calculated based on the measured apparent ellipticity according to reported formulas: Ellipticity ([θ] in deg cm^2 dmol^{-1}) = (millidegrees × mean residue weight)/(path length in millimeters × concentration of polypeptide in mg/mL).[2] The helicity of the polypeptide was calculated according to the following equation: Helicity = (−[θ_{222}] + 3,000)/39,000. Dynamic light scattering (DLS) analysis was conducted on a Zetasizer Nano ZS90 (Malvern Instruments, Ltd., UK) with a He-Ne laser (λ = 633 nm) at a scattering angle of 90^o (25 ℃).

Synthesis of PPOBLG.

The polypeptide PPOBLG was synthesized via ring-opening polymerization (ROP) of POBLG-NCA as initiated by *n*-butylamine. Briefly, POBLG-NCA (0.86 g, 2.71 mmol) was dissolved in anhydrous DMF (5 mL) in a vial under nitrogen, into which a solution of *n*-butylamine in DMF (0.1 mol/L, 271 μL, 0.027 mmol) was added with a syringe. The reaction mixture was stirred at room temperature for 72 h. The product PPOBLG was precipitated with distilled water (60 mL) and collected as white solid (700 mg, yield 94%).

Synthesis of *n*F-Cl (*n* = 3 and 5).

6-Chlorohexanol (1.00 g, 7.32 mmol), trifluoroacetic anhydride (2.00 g, 9.51 mmol), pyridine (1.74 g, 21.96 mmol), and DMAP (60 mg, 0.488 mmol) were mixed in a 10-mL vial in ice-water bath, and the solution was stirred at room temperature for 84 h. The solution turned yellow and precipitates appeared during this process. The mixture was dissolved in dichloromethane (50 mL), followed by washing with brine (50 mL × 1), 1 M HCl (50 mL × 4), and brine (50 mL × 2). The organic phase was dried over anhydrous sodium sulfate and filtered to afford 3F-Cl as light yellow liquid after removal of the solvent under vacuum (yield 83%).

5F-Cl was prepared from 6-chlorohexanol (1.00 g, 7.32 mmol), pentafluoropropionic anhydride (2.72 g, 8.78 mmol), pyridine (1.74 g, 21.96 mmol), and DMAP (60 mg, 0.488 mmol) using the same procedure as for 3F-Cl (yield 86%).

Synthesis of 7F-Cl and 7H-Cl.

6-Chlorohexanol (1.00 g, 7.32 mmol), heptafluorobutyric anhydride (3.60 g, 8.78

mmol), pyridine (1.74 g, 21.96 mmol), and DMAP (60 mg, 0.488 mmol) were mixed in a 10-mL vial in an ice-water bath, and the solution was stirred at room temperature for 84 h. The solution turned yellow and precipitates appeared during this process. The precipitate was dissolved in dichloromethane (50 mL), followed by washing with brine (50 mL × 1), 1 M HCl (50 mL × 4), and brine (50 mL × 2). The solvent was removed by vacuum and crude product was obtained as light yellow oil. To further remove pentafluoropropionic anhydride, distilled water (20 mL) was added to the crude product, and the mixture was stirred at 60 ^{o}C for 1 h. Then the mixture was dissolved in dichloromethane (50 mL), followed by washing with saturated $NaHCO_3$ solution (50 mL × 2) and brine (50 mL × 2). The organic phase was dried over anhydrous sodium sulfate and filtered to afford 7F-Cl as light yellow liquid after removal of the solvent under vacuum (yield 79%).

7H-Cl was prepared from 6-chlorohexanol (1.00 g, 7.32 mmol), butyric anhydride (1.19 g, 7.47 mmol), pyridine (1.74 g, 21.96 mmol), and DMAP (60 mg, 0.488 mmol) using the same procedure as for 7F-Cl (yield 74%).

Synthesis of *n*F-N_3 (*n* = 3, 5, 7) and 7H-N_3.

3F-Cl (0.8 g, 3.44 mmol) and NaN_3 (1.12 g, 17.19 mmol) were dissolved in DMF (2 mL), and the mixture was stirred at 60 ^{o}C for 48 h. The mixture was dissolved in hexane (50 mL), followed by washing with brine (50 mL × 4) and drying over anhydrous sodium sulfate. After filtration and removal of the solvent under vacuum, 3F-N_3 was obtained as transparent liquid (yield 76%).

5F-N_3, 7F-N_3, and 7H-N_3 were prepared from 5F-Cl, 7F-Cl, and 7H-Cl,

respectively, using the same procedure as for 3F-N_3 (yield 78%, 81%, and 75%, respectively).

Synthesis of P*m*F*x*, P7H7, and PG1.

Fluorinated polypeptides and the non-fluorinated polypeptides (PG1 and P7H7) were prepared via side-chain functionalization of PPOBLG using click chemistry (Table S3). PPOBLG, 6-azidohexyl guanidine, and *n*F-N_3 (or 7H-N_3) were dissolved in DMF (3 mL) in a glass vial (10 mL) in the glovebox, followed by the addition of N,N,N′,N″,N″-pentamethyldiethylene-triamine (PMDETA, 34 μL, 0.125 mmol) and CuBr (18 mg, 0.125 mmol). The reaction solution was stirred at room temperature for 24 h and quenched by exposure to air. HCl (1 M, 3-4 mL) was then added until the solution became colorless. The product was dialyzed against distilled water (MWCO = 3500 Da) for three days and lyophilized to afford white solid.

Table S3. Synthesis of non-fluorinated and fluorinated polypeptides from PPOBLG.

	PPOBLG (mg)	G-6-N_3 (mg)	*n*F-N_3 (mg)	7H-N_3 (mg)	Yield (%)
PG1	30	22.3	-	-	91
P3F7	30	20.7	2.1	-	94
P3F16	30	18.9	4.3	-	87
P3F31	30	15.6	8.7	-	86
P5F6	30	20.7	2.5	-	90
P5F16	30	18.9	5.3	-	90
P5F34	30	15.6	10.5	-	88
P7F7	30	20.7	2.9	-	92
P7F18	30	18.9	6.2	-	85
P7F34	30	15.6	12.3	-	87
P7H7	30	20.7	-	1.8	90

Characterization of polypeptide/siRNA polyplexes.

Polypeptide (0.2 mg/mL) and siRNA (0.1 mg/mL) were separately dissolved in DEPC-treated water. The polypeptide solution was added into the siRNA solution at various weight ratios, and the polypeptide/siRNA polyplexes were formed after vortex for 10 s and incubation at 37 ^{o}C for 30 min. The siRNA condensation level of polyplexes was assessed by using a gel retardation assay via electrophoresis on 2% agarose gel at 90 V for 20 min. An ethidium bromide (EB) exclusion assay was further performed to quantitatively monitor the siRNA condensation level as reported before.[3] The particle size and zeta potential of polypeptide/siRNA polyplexes were measured by DLS.

Cytotoxicity of polypeptides.

RAW 264.7 cells (~70% confluence in 96-well plates) were treated with PG1/siNC polyplexes or P*m*F*x*/siNC polyplexes (w/w = 15) at the polymer concentration of 15 μg/mL in serum-free DMEM for 4 h. The medium was replaced by DMEM containing 10% FBS, and cells were further incubated for 20 h before assessment of cell viability by the MTT assay. Results were presented as percentage viability of control cells that did not receive treatment with polyplexes.

Cell uptake of polyplexes.

RAW 264.7 cells (~70% confluence in 96-well plates) were incubated with polypeptide/Cy3-siRNA polyplexes (w/w = 15) at 1 μg Cy3-siRNA/mL in serum-free DMEM for 4 h. Cells were then washed with cold PBS containing heparin (20 U/mL) for three times and lysed with the RIPA lysis buffer (100 μL/well). Cy3-siRNA content in the lysate was monitored by spectrofluorimetry (λ_{ex} = 550 nm, λ_{em} = 565

nm) and protein level was quantified using the BCA kit. Uptake level was expressed as μg Cy3-siRNA associated with 1 mg of cellular protein.

Intracellular distribution.

RAW 264.7 cells (~20% confluence in a culture dish, Φ = 15 mm) were incubated with polypeptide/FAM-siRNA polyplexes (w/w = 15) at 0.5 μg/mL FAM-siRNA in serum-free DMEM for 4 h. Cells were then washed with cold PBS containing heparin (20 U/mL) for three times, stained with Hoechst 33258 (5 μg/mL) for 30 min and Lysotracker Red (200 nM) for 1 h, and observed by confocal laser scanning microscopy (CLSM).

***In vitro* TNF-α knockdown efficiency.**

RAW 264.7 cells (~70% confluence in 96-well plates) were treated with polypeptide/siTNF-α polyplexes (w/w = 15) at 1 μg/mL siRNA in serum-free DMEM for 4 h. The medium was then replaced by fresh DMEM containing 10% FBS, and cells were further cultured for another 20 h before challenge with LPS (7.5 ng/mL) for 5 h. The TNF-α level in the medium was determined by ELISA, and the knockdown efficiency was denoted as the percentage TNF-α level of control cells that did not receive polyplexes treatment. PEI/siTNF-α polyplexes (w/w = 5) were used as a commercial control. The TNF-α mRNA level was determined by real-time PCR. Cells were treated with polyplexes and challenged with LPS as described above. Five hours later, total RNA was isolated from cells using the Trizol reagent, and cDNA was synthesized from total RNA using the high-capacity cDNA reverse transcription kit according to the manufacturer's instructions. Synthesized cDNA, TNF-α primers, and

SYBR Premix Ex Taq were mixed and run on the real-time PCR system (Bio-Rad CFX connect). All samples were analyzed for 36B4 expression in parallel in the same run. Results were expressed as percentage TNF-α mRNA level of control cells that were challenged by LPS but not treated with polyplexes.

***In vitro* permeation across Calu-3 cell monolayers**

Air-interfaced culture (AIC) of Calu-3 cells, a well-established *in vitro* model of bronchial epithelia with secreted mucus layers,[4] was adopted herein to evaluate the mucus/epithelia penetration capabilities of polyplexes. Briefly, Calu-3 cells were seeded on Transwells (0.33 cm^2, pore size of 3.0 μm, Corning, NY) at 5.0×10^5 cell/cm^2 and were cultured for 14 days. The transepithelial electrical resistance (TEER) was daily measured during day 7 to 14. During the cells culture, the medium in the apical compartment needed to be removed at 4 days post cell seeding while the medium in the basolateral side needed to be replaced every day. When the TEER value reached 700 Ω·cm^2 (usually within 14 days), the cell monolayers could be used for the permeation studies before washing with PBS for three times and addition of Hank's balanced salt solution (HBSS, 500 μL) containing 1% BSA to the basolateral side. Polypeptide/Cy3-siRNA polyplexes (w/w = 15, 2 μg Cy3-siRNA) were then added to the apical side in 200 μL of HBSS containing 1% BSA. After incubation at 37 ℃ for 6 h, the medium in the basolateral side was harvested, and the amount of Cy3-siRNA was determined by spectrofluorimetry. The penetrating capabilities of the polyplexes were represented by the apparent permeability coefficient (P_{app}) using the equation of $P_{app} = Q/Act$, where Q is the amount of permeated Cy3-siRNA (ng), A is

the diffusion area of the cell monolayers (cm^2), *c* is the initial concentration of Cy3-siRNA at the apical side (ng/cm^3), and *t* is the transport time (s).

Multiple particle tracking in CF mucus.

CF mucus was obtained from CF patients of the Second Affiliated Hospital of Soochow University, and was diluted with PBS for 20 fold before use. The multiple particle tracking technology was used to determine the trajectory of polypeptide/siRNA polyplexes in the CF mucus.[5] Briefly, polypeptide/Cy3-siRNA polyplexes (w/w = 15, with 2 μg Cy3-siRNA) were mixed with the CF mucus (1 mL) and transferred to a 8-well chamber slide. After equilibration for 1 h at 37 ℃, twenty-second movies at 66.7 ms temporal resolution were acquired using an Evolve 512 EMCCD camera (Photometrics, Tucson, AZ) equipped on an inverted epifluorescence microscope (Observer Z1, Zeiss; Thornwood, NY) with a 100×1.4 NA objective. Movies were analyzed with the Imaris software to extract movement orbits and mean square displacement (MSD) for individual polyplexes.

Stability of polyplexes in CF mucus.

PPOBLG (10 mg) was labeled with Cy5-NHS (0.5 mg) in DMF (2 mL) in the dark overnight via reaction of the terminal amine group on the polypeptide backbone with Cy5-NHS. Fluorocarbon and guanidine groups were then incorporated onto side chains via click chemistry as mentioned above. Cy5-polypeptide/Cy3-siRNA polyplexes (w/w = 15) were prepared using the same method as described above, and polyplexes containing 5 μg Cy3-siRNA were incubated with the CF mucus (2 mL) for 30 min at 37 ℃. The fluorescence intensity at 667 and 568 nm upon excitation at 550

nm (for Cy3) was measured by recording the fluorescence emission spectra according to the previous literature.[6]

Mucin aggregation study.

For the measurement of polyplexes-mucin interaction, polypeptide/Cy3-siRNA polyplexes (w/w = 15, 5 μg siRNA) were mixed with mucin solution (0.3% or 0.5% in PBS, 2 mL), vortexed, and incubated at 37 ^{o}C for 30 min in a shaker. The mixture was centrifuged at 1500 rpm for 2 min and the precipitates were washed twice with PBS. Then, the precipitates were treated with NaOH (5 M, 200 μL) for 10 min, and the fluorescence intensity was measured (λ_{ex} = 550 nm, λ_{em} = 565 nm).[7]

***In vivo* mucus penetration and distribution in lung tissues.**

Male Balb/c mice were intratracheally injected with LPS solution (5 mg/mL in saline, 50 μL) to induce ALI. At 2 h post LPS challenge, polypeptide/Cy3-siRNA polyplexes (w/w =15) were intratracheally injected at 200 μg siRNA/kg. Two hours after injection, animals were sacrificed and lung tissues were collected, fixed in 4% paraformaldehyde, dehydrated in saturated sucrose solution, embedded in OCT, and cryo-sectioned at the thickness of 5 μm. The sections were stained with DAPI (for cell nuclei), and were then observed by CLSM. Ten confocal images were taken for each experiment and the most representative image was selected.

***In vivo* gene knockdown efficiency and anti-inflammatory efficacy.**

Male Balb/c mice were randomly divided into six groups, and animals in groups 1-5 received intratracheal injection of LPS (5 mg/mL in saline, 50 μL) to induce ALI. Two hours later, animals in groups 1-5 received intratracheal injections of PBS,

PG1/siTNF-α polyplexes (w/w = 15), P3F16/siTNF-α polyplexes (w/w = 15), P7F7/siTNF-α polyplexes (w/w = 15), and PEI/siTNF-α polyplexes (w/w = 5) at 200 μg siRNA/kg. Mice in group 6 did not receive LPS or polyplexes administration and thus served as the normal control. Mice were sacrificed after another 22 h, and lung tissues were harvested for the following analyses. TNF-α mRNA level in the lung tissues was monitored by real-time PCR. The lung tissues were homogenized with the Trizol reagent to isolate total RNA; the synthesis of cDNA and real-time PCR analysis were performed as described above. The TNF-α and IL-6 protein levels in lung tissues were evaluated by ELISA and Western blot after the lung tissues were homogenized with the RBC lysis buffer.

The myeloperoxidase (MPO) level in the lung tissues was also measured to indicate neutrophil infiltration. Briefly, lung tissues were homogenized with the passive lysis buffer, and the homogenate was centrifuged at 13,000 rpm and 4 ^{o}C for 10 min before quantification of the MPO activity in the supernatant using the MPO detection kit.

Bronchoalveolar lavage fluid (BALF) collection and analysis.

The BALF was collected at 22 h post polyplexes administration, and it was centrifuged for 10 min at 15,000 rpm and 4 ^{o}C. The supernatant was collected and subjected to the measurement of TNF-α and IL-6 levels using the ELISA kit and total protein level using the BCA kit. The cell pellets were re-suspended in PBS to allow for total and differential cell counting.

Measurement of wet/dry weight ratios of lung tissues.

The water content in the lung tissues was determined by calculating the wet/dry weight ratio. At 22 h post intratracheal administration of the polyplexes, the right cranial lobe was excised, washed with PBS, blotted, and weighed to obtain the "wet" weight. The lung tissue was then dried at 80 °C for 72 h to obtain the "dry" weight, and the wet/dry weight ratio was accordingly calculated.

HE staining.

The lung tissues were harvested at 22 h post intratracheal administration of the polyplexes, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 8-μm thickness, and stained with hematoxylin/eosin (HE) before histological observation using optical microscopy.

Blood gas analysis.

Blood samples were obtained from the arteria carotis at 22 h post intratracheal administration of the polyplexes, and were directly subjected to the measurement of partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), and pH by using the blood-gas analyzer (Radiometer, Shanghai, China).

Statistical analysis.

Statistical analysis was conducted using the Student's t test, and differences were assessed to be significant at $*p < 0.05$ and very significant at $**p < 0.01$ and $***p < 0.001$.

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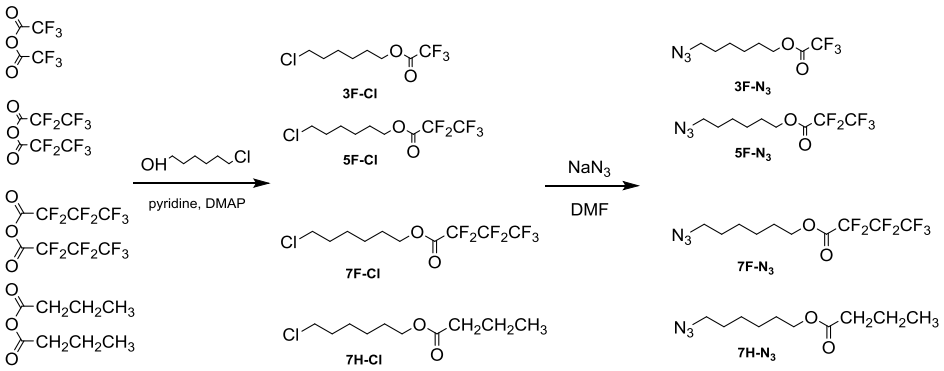
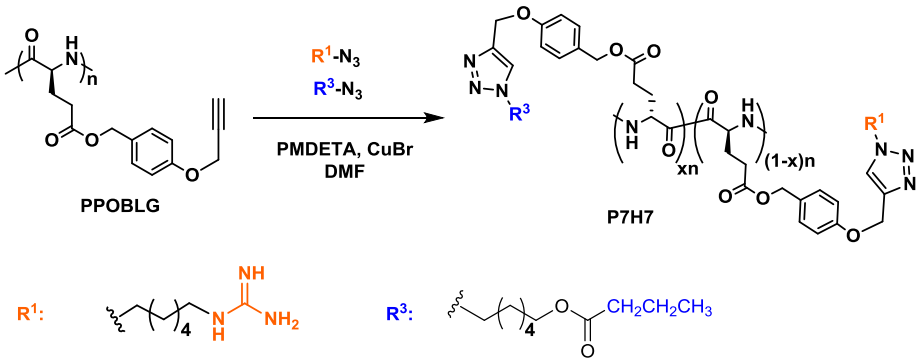


Scheme S1. Synthetic routes of 3F-N_3, 5F-N_3, 7F-N_3, and 7H-N_3.



Scheme S2. Synthetic route of P7H7.

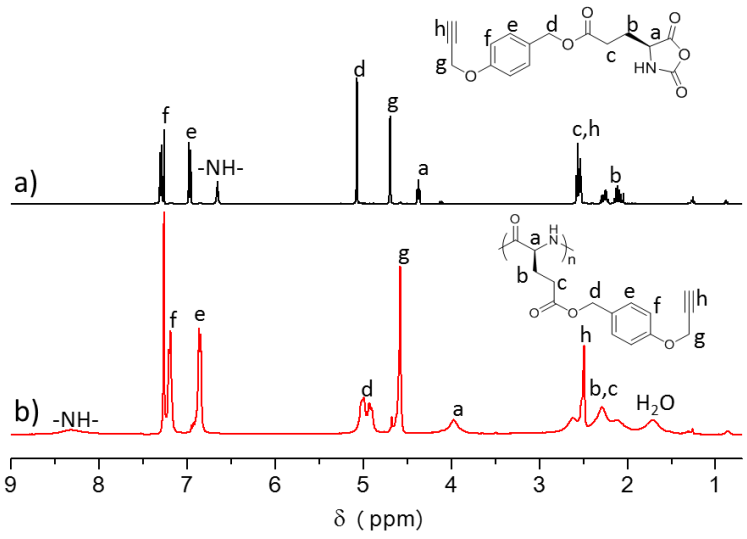


Figure S1. ^{1}H NMR spectra of POBLG-NCA a) and PPOBLG b) in $CDCl_3$.

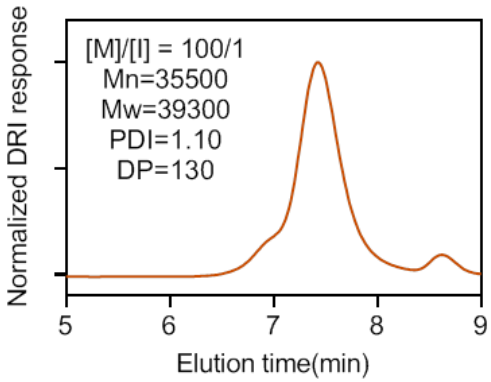


Figure S2. GPC curve of PPOBLG using DMF containing 0.05 M LiBr as the eluent phase at a flow rate of 1.0 mL/min.

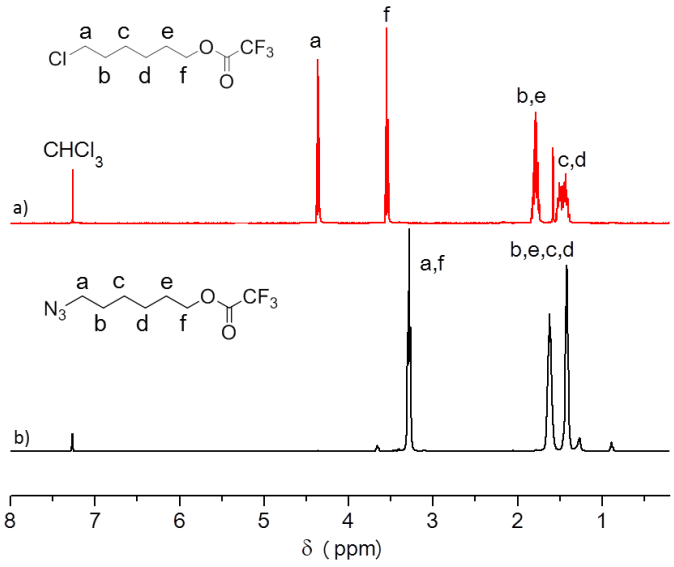


Figure S3. ^{1}H NMR spectra of 3F-Cl a) and 3F-N_3 b) in $CDCl_3$.

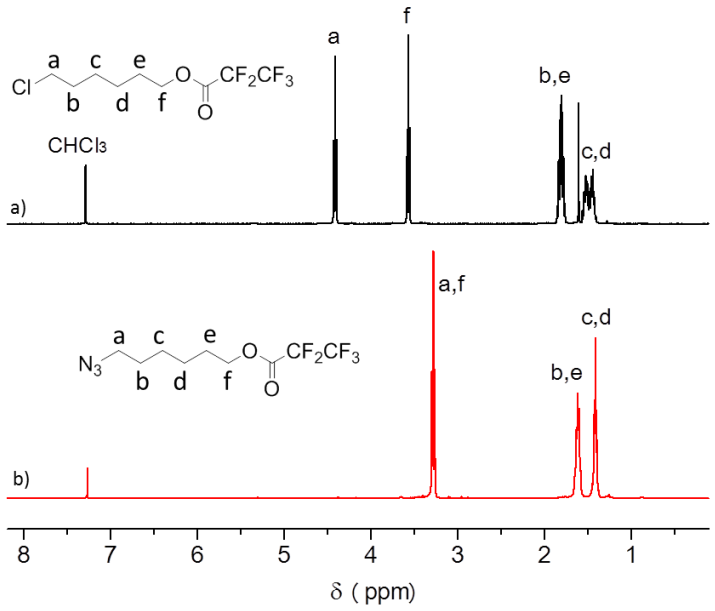


Figure S4. ^{1}H NMR spectra of 5F-Cl a) and 5F-N_3 b) in $CDCl_3$.

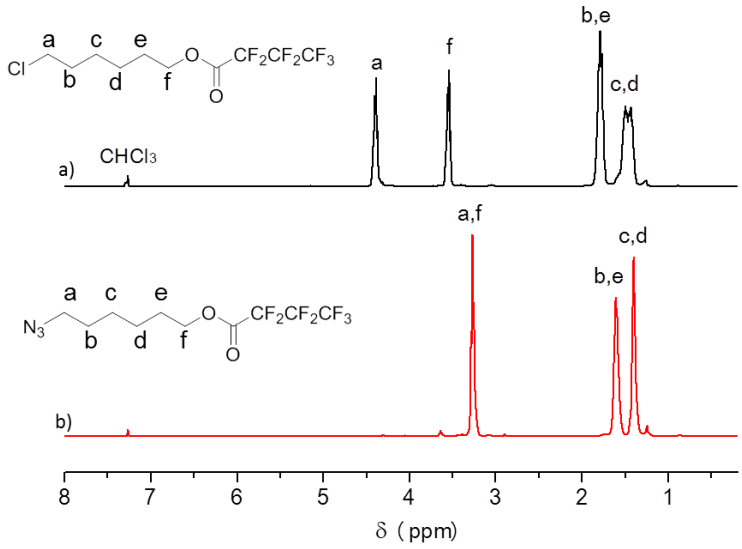


Figure S5. ^{1}H NMR spectra of 7F-Cl a) and 7F-N_3 b) in $CDCl_3$.

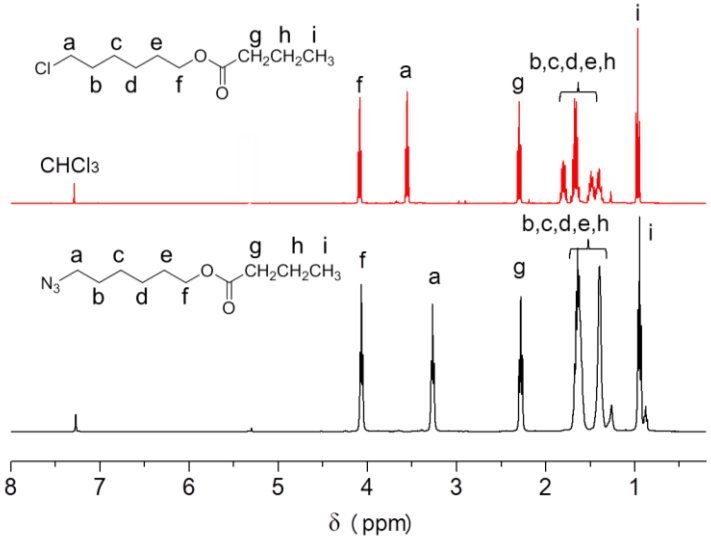


Figure S6. ^{1}H NMR spectra of 7H-Cl a) and 7H-N_3 b) in $CDCl_3$.

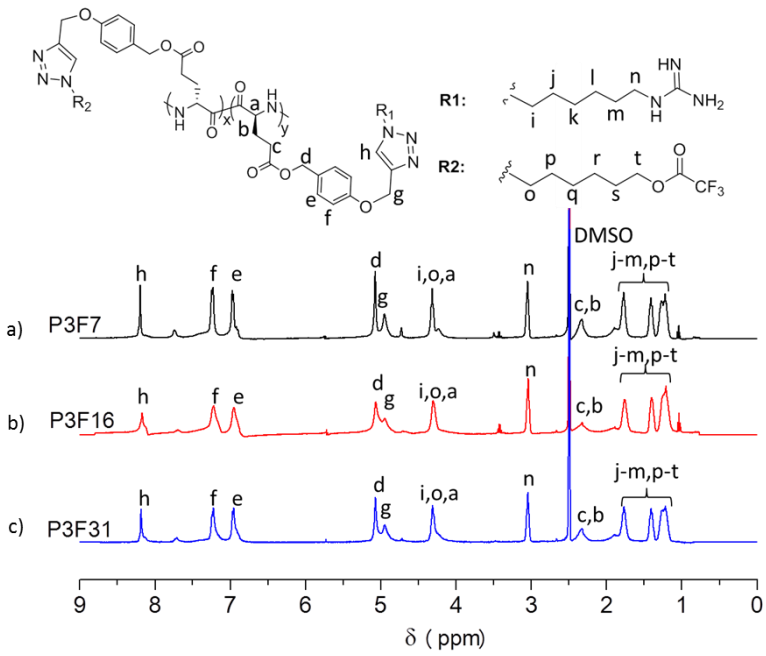


Figure S7. ^{1}H NMR spectra of P3F7 a), P3F16 b), and P3F31 c) in DMSO-d_6/TFA-d (9/1, v/v).

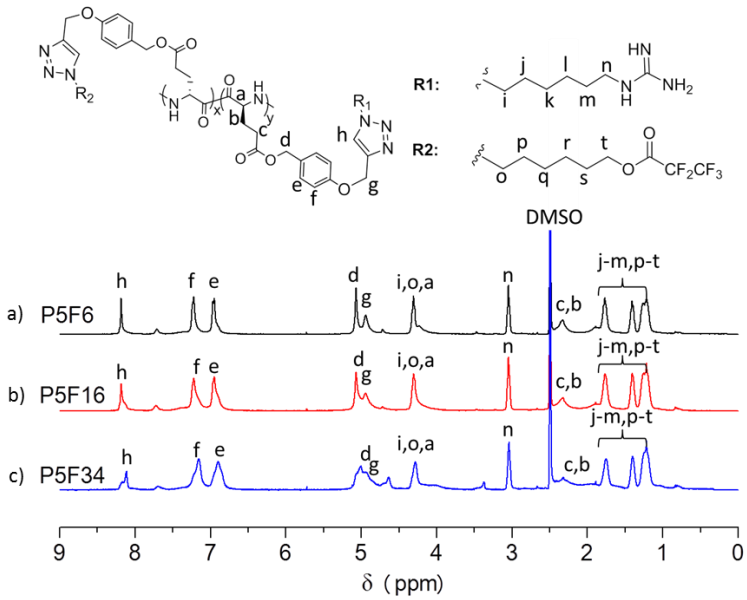


Figure S8. [1]H NMR spectra of P5F6 a), P5F16 b), and P5F34 c) in DMSO-d_6/TFA-d (9/1, v/v).

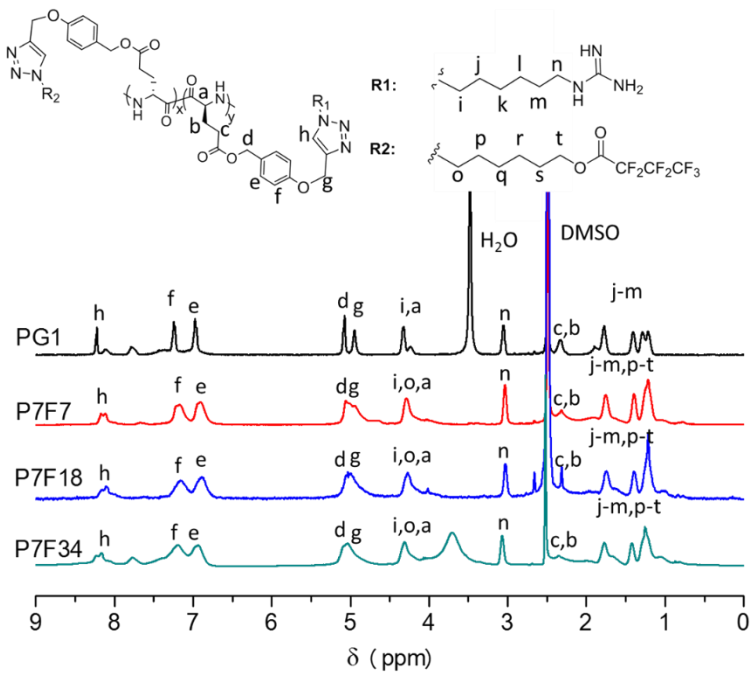


Figure S9. [1]H NMR spectra of PG1 a), P7F7 b), P7F18 c), and P7F34 d) in DMSO-d_6/TFA-d (9/1, v/v).

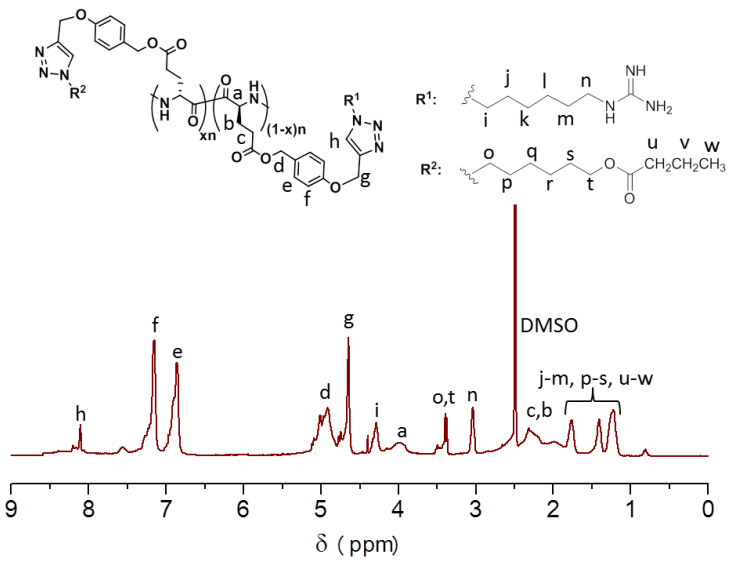


Figure S10. ^{1}H NMR spectrum of P7H7 in DMSO-d_6/TFA-*d* (9/1, v/v).

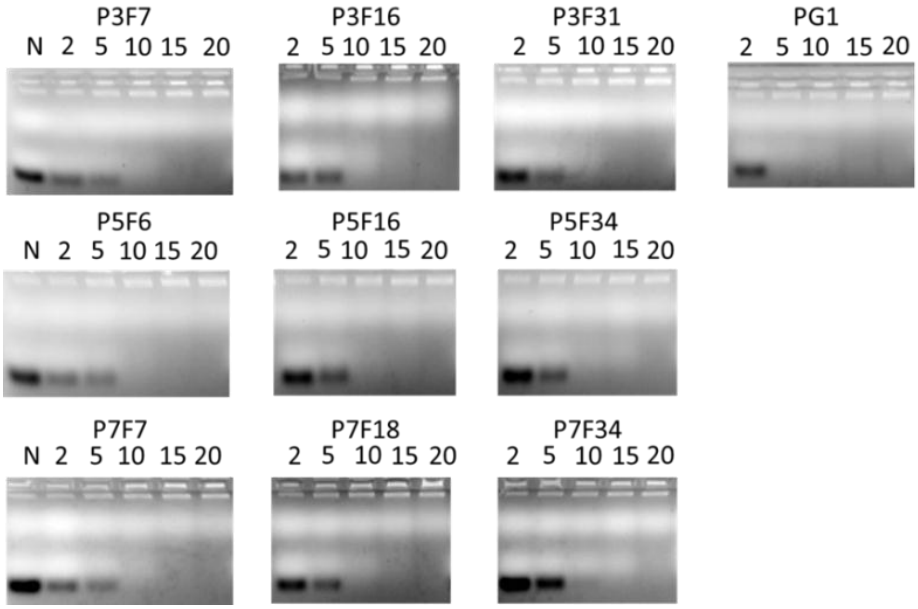


Figure S11. siRNA condensation by polypeptides at various polypeptide/siRNA weight ratios as evaluated by the gel retardation assay. N represents naked siRNA.

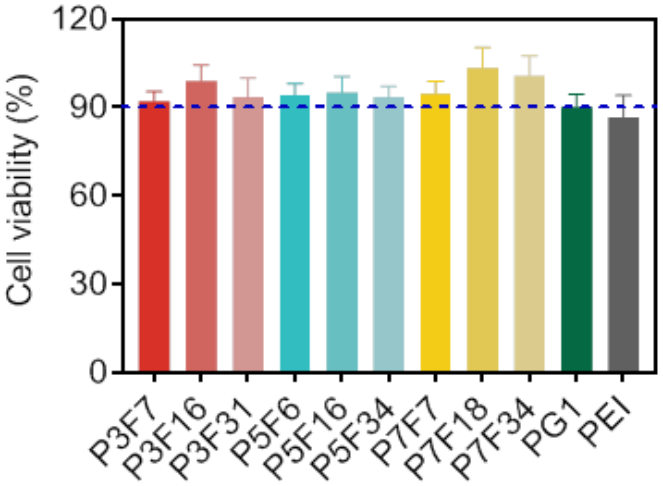


Figure S12. Cytotoxicity of polypeptide/siNC polyplexes (w/w = 15) and PEI/siNC polyplexes (w/w = 5) at the polymer concentration of 15 μg/mL in RAW 264.7 cells (n = 3).

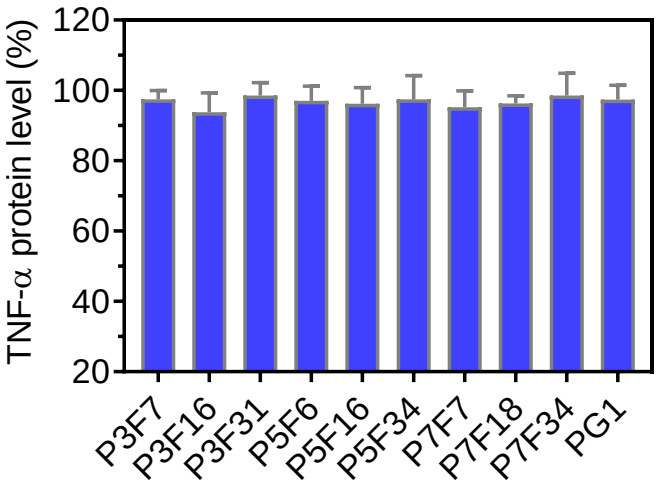


Figure S13. TNF-α secretion levels as determined by ELISA (n = 3). Cells were incubated with polyplexes (1 μg/mL siNC) for 4 h, cultured in fresh medium for 20 h, stimulated with LPS (7.5 ng/mL) for 5 h, and subjected to the determination of TNF-α levels.

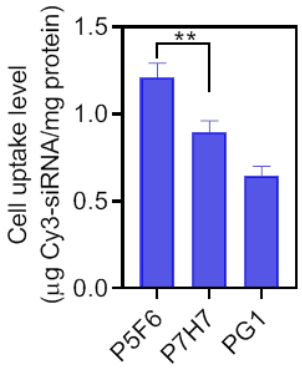


Figure S14. Uptake levels of polypeptide/Cy3-siRNA polyplexes (w/w = 15) in RAW 264.7 cells following 4-h incubation at 1 μg/mL siRNA (n = 3).

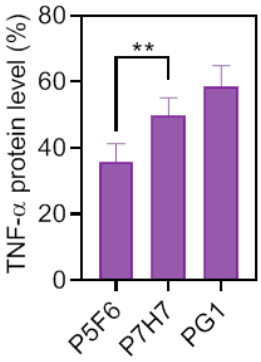


Figure S15. TNF-α knockdown efficiencies of polypeptide/siTNF-α polyplexes (n = 3). Cells were incubated with polyplexes (1 μg/mL siRNA) for 4 h, cultured in fresh medium for 20 h, stimulated with LPS (7.5 ng/mL) for 5 h, and subjected to the determination of TNF-α levels by ELISA.

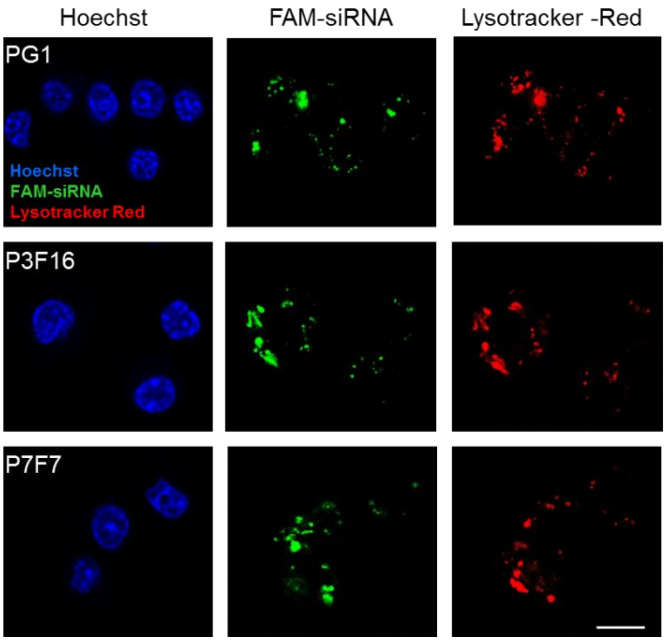


Figure S16. CLSM images of RAW 264.7 cells treated with PG1/FAM-siRNA, P3F16/FAM-siRNA, and P7F7/FAM-siRNA polyplexes (w/w = 15) for 4 h. Cell nuclei were stained with Hoechst 33258, and endosomes/lysosomes were stained with Lysotracker Red (scale bar = 20 μm).

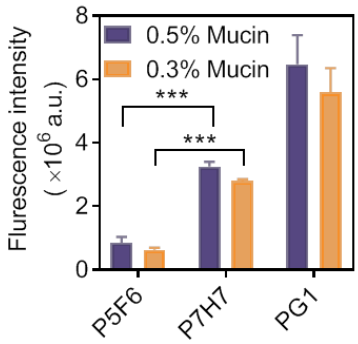


Figure S17. The fluorescent intensity of polypeptide/Cy3-siRNA polyplexes-mucin aggregates at different mucin concentrations (n = 3).

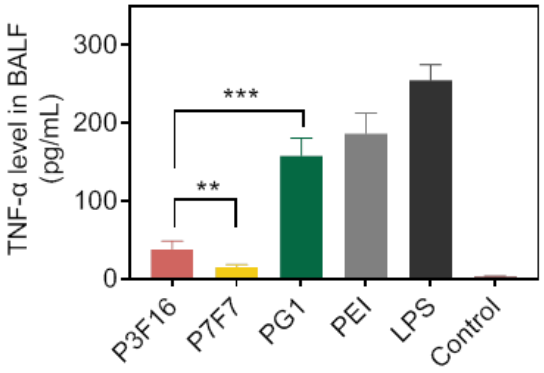


Figure S18. TNF-α levels in BALF harvested from mice stimulated with LPS and intratracheally administered with polyplexes (200 μg siTNF-α/kg) (n = 6).

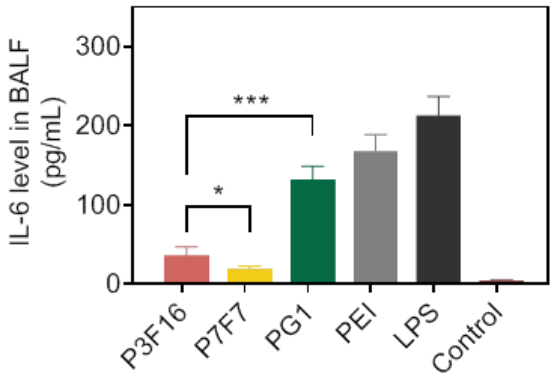


Figure S19. IL-6 levels in BALF harvested from mice stimulated with LPS and intratracheally administered with polyplexes (200 μg siTNF-α/kg) (n = 6).

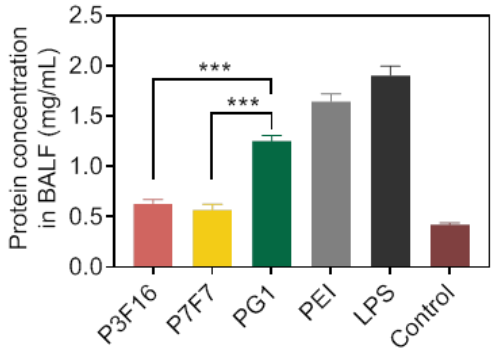


Figure S20. Protein concentration in BALF harvested from mice stimulated with LPS and intratracheally administered with polyplexes (200 μg siTNF-α/kg) (n = 6).

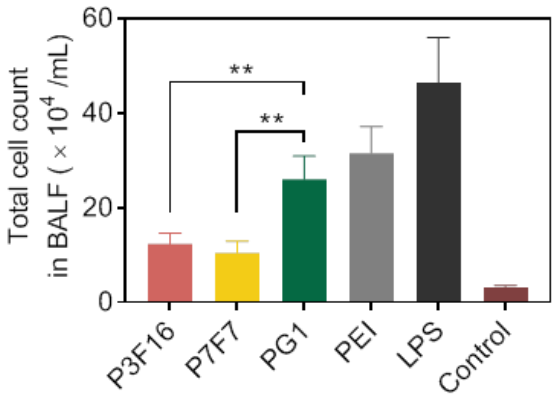


Figure S21. Total cell count in BALF harvested from mice stimulated with LPS and intratracheally administered with polyplexes (200 μg siTNF-α/kg) (n = 6).