Supporting Information

Efficient pDNA Delivery Using Cationic 2-Hydroxypropyl-β-Cyclodextrin:Pluronic-based Polyrotaxanes

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1. Synthesis of Bis-Cholesterol-End-Capped HPCD:Pluronic Polyrotaxanes

Typically, dried α, ω-bis-tris (2-aminoethyl) amine Pluronic triblock copolymer (0.08 mmol) and 2-hydroxypropyl-β-cyclodextrin (using the ratio of CD:PPG unit = 1:2 for each type of Pluronic) were dissolved (or suspended) in 60 mL hexane and the mixtures were vortexed before vigorously stirring for 2 h. Then, bath sonication for 1 h at 20 °C, followed by 10 min probe sonication (Model W-350, 50 W, ½" probe) was performed to improve the threading efficiency of the Pluronic copolymer. The mixtures were then stirred for 72 h at 20 °C before removing the solvent under reduced pressure. The mixtures were redissolved in 20 mL of dried CH_2Cl_2 and cholesteryl chloroformate (12 equiv) was added. The reaction mixtures were stirred at 20 °C for 24 h and concentrated before precipitation in diethyl ether (700 mL). To remove unreacted reagents and unthreaded cyclodextrins, the crude products were dissolved in CH_3OH (20 mL), precipitated in 500 mL diethyl ether, and filtered. Finally, the products were purified by sequential dialysis using 12,000-14,000 and 6,000-8,000 MWCO regenerated cellulose membranes in DMSO first and progressively exchanged with deionized water for 5 days before lyophilization to generate white HPCD:Pluronic PR^+ powders. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.92 ppm (S, H-NCO carbamate), 5.25 ppm (t, 1H, Chl-ethylene H), 5.0 ppm (b, C_1-H of CD), 4.5 ppm (b, OH propyl), 3.5-3.8 ppm (m, $C_{3,5,6}$-H of CD), 3.5 ppm (m, PEG-CH_2), 2.6-2.8 ppm (m, 16H, CH_2 of TAEA), 1.0 ppm (d, CH_3 of PPG), 0.8-0.6 ppm (m, Chl-CH_3).

^{1}H NMR Spectrum of HPCD:F127 Pluronic PR

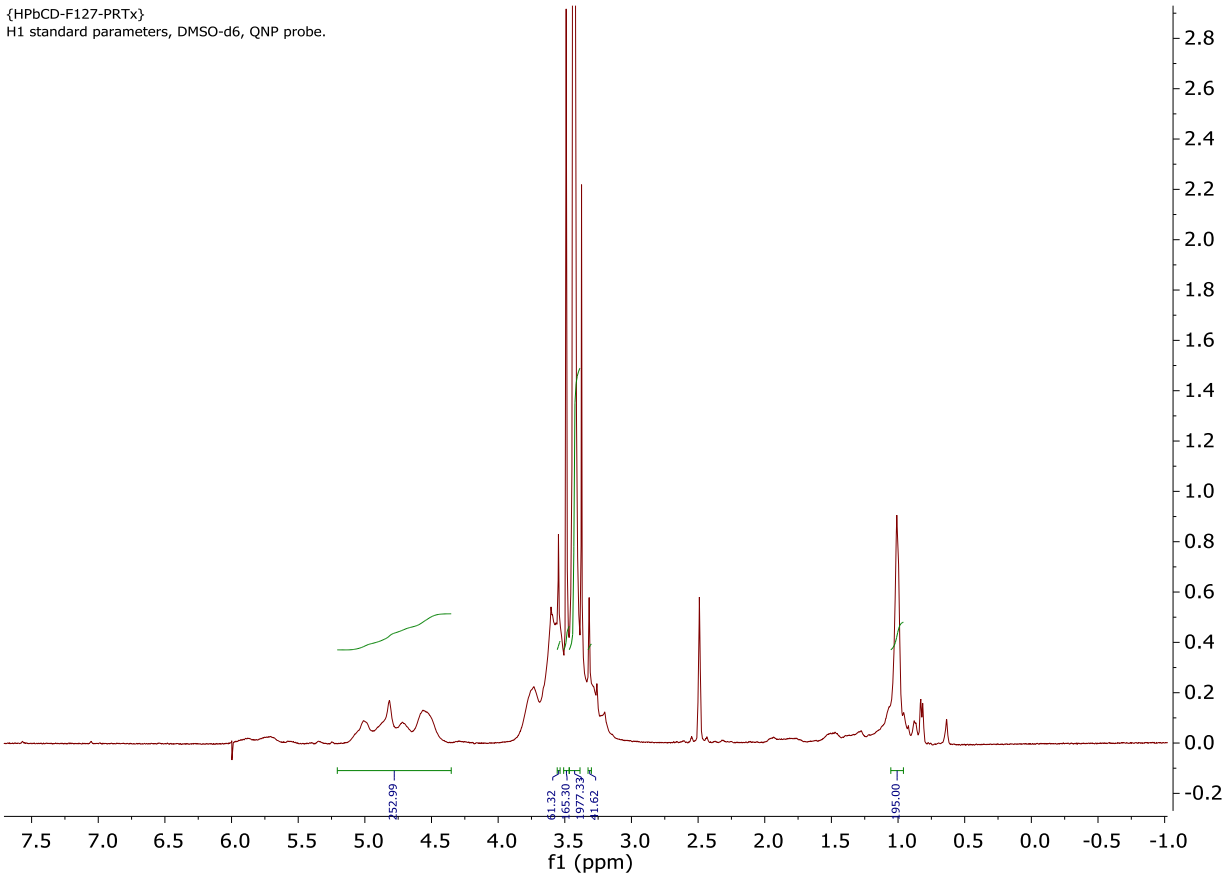

Figure S1: ^{1}H NMR Spectrum of HPCD:F127 Pluronic PR

^{1}H NMR Spectrum of HPCD:F68 Pluronic PR

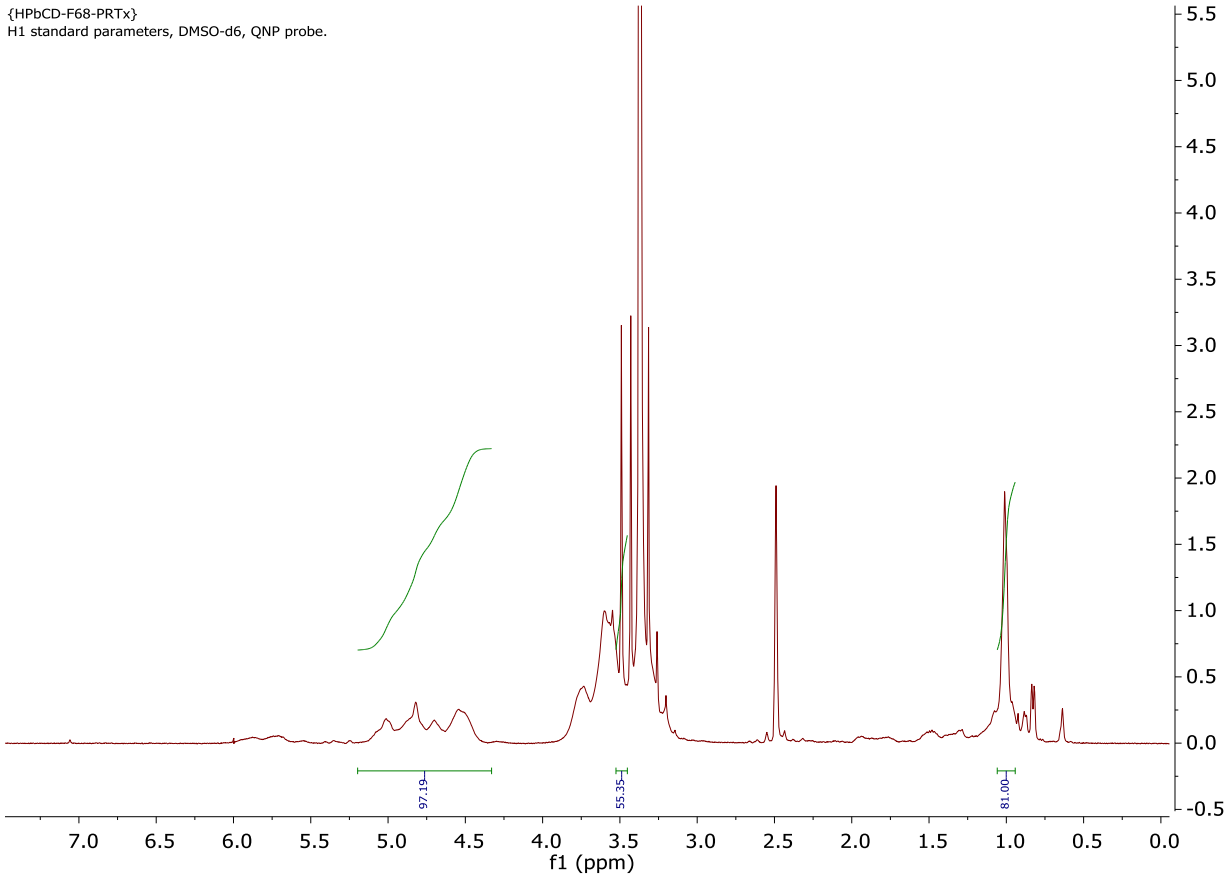

Figure S2: ^{1}H NMR Spectrum of HPCD:F68 Pluronic PR

[1]H NMR Spectrum of HPCD:L35 Pluronic PR

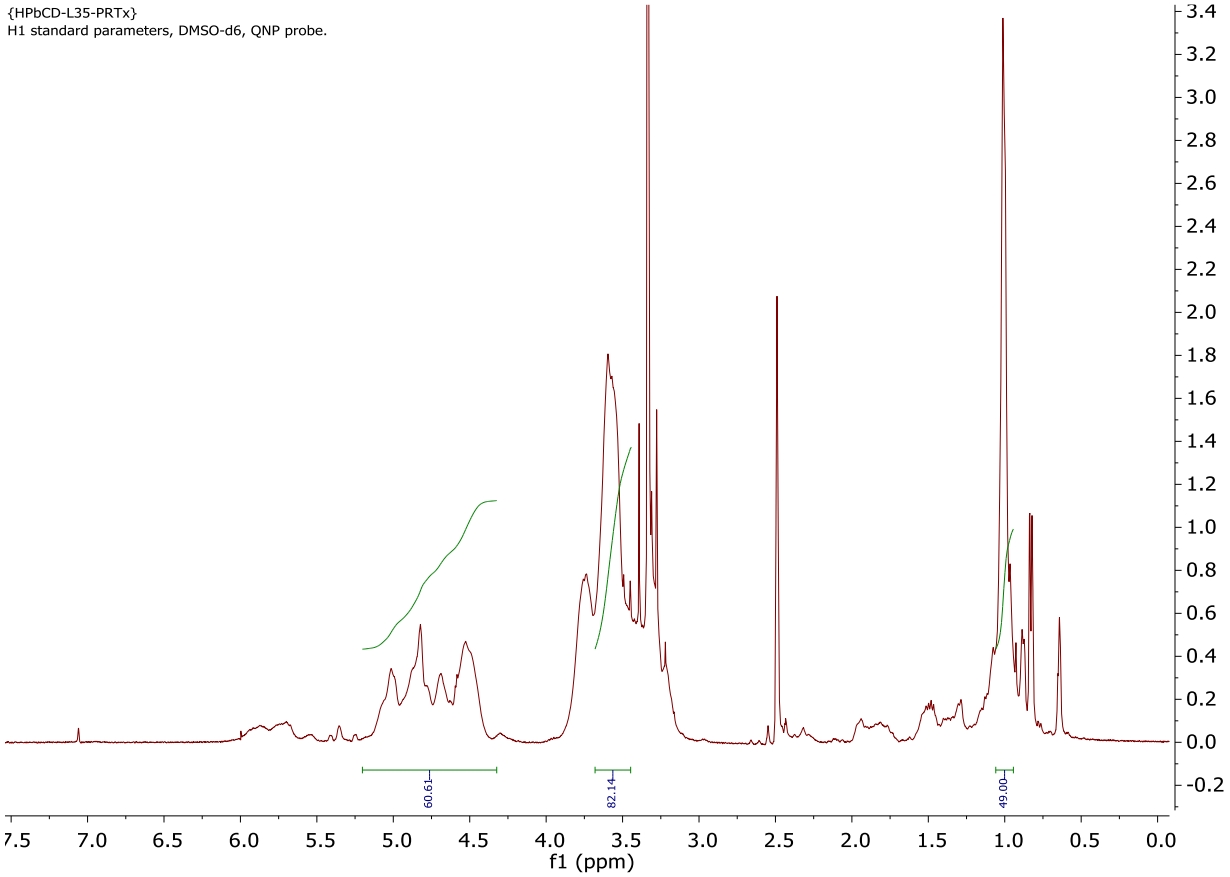

Figure S3: [1]H NMR Spectrum of HPCD:L35 Pluronic PR

^{1}H NMR Spectrum of HPCD:L64 Pluronic PR

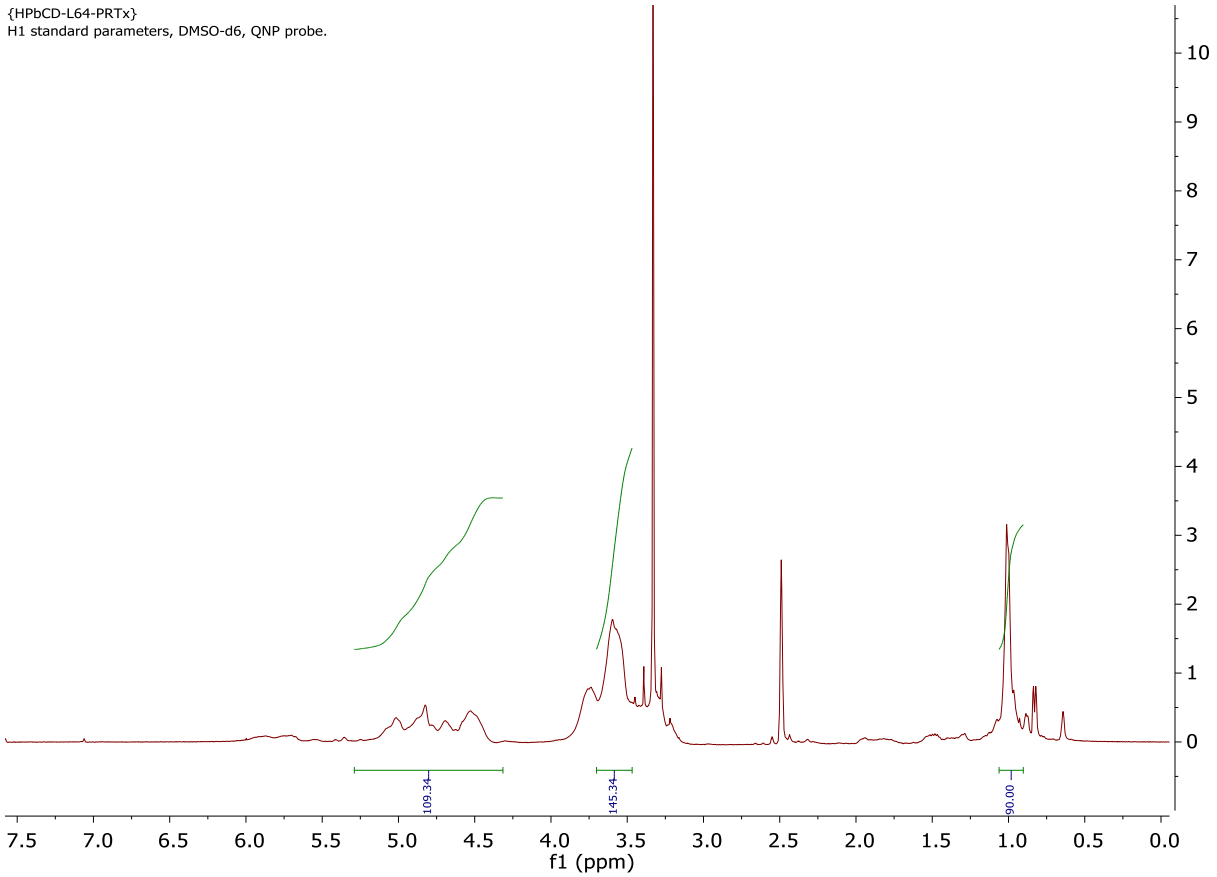

Figure S4: ^{1}H NMR Spectrum of HPCD:L64 Pluronic PR

^{1}H NMR Spectrum of HPCD:L81 Pluronic PR

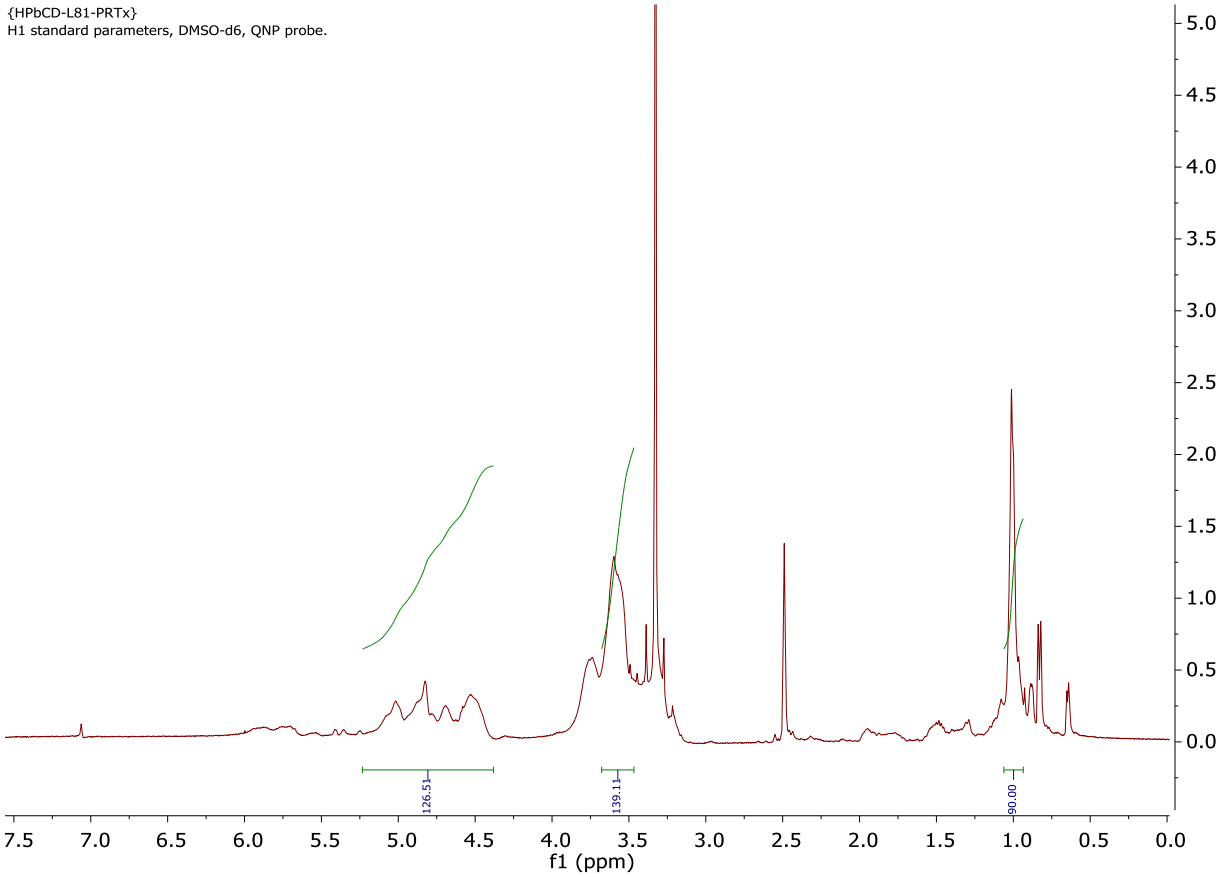

Figure S5: ^{1}H NMR Spectrum of HPCD:L81 Pluronic PR

^{1}H NMR Spectrum of DMEDA HPCD:F127 Pluronic PR

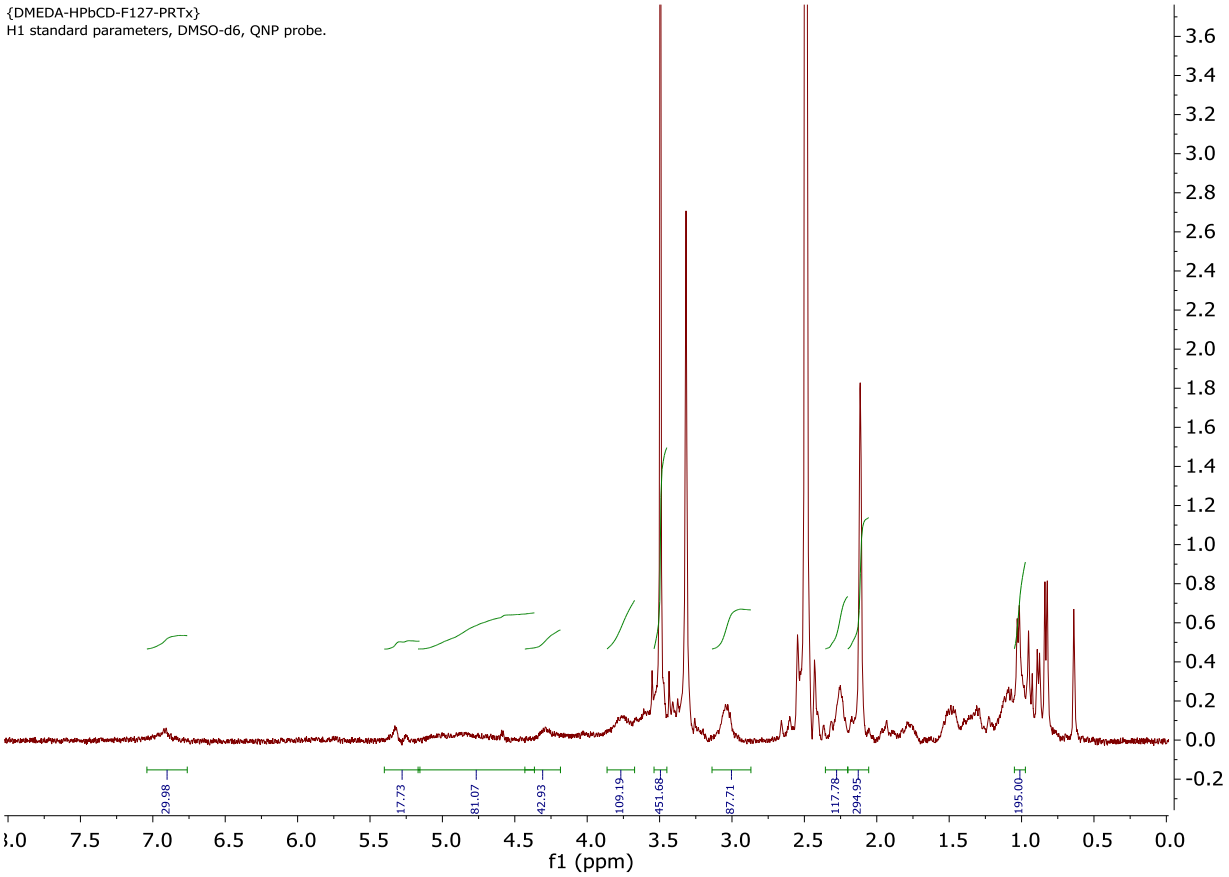

Figure S6: ^{1}H NMR Spectrum of DMEDA-HPCD:F127 Pluronic PR

^{1}H NMR Spectrum of DMEDA-HPCD:F68 Pluronic PR

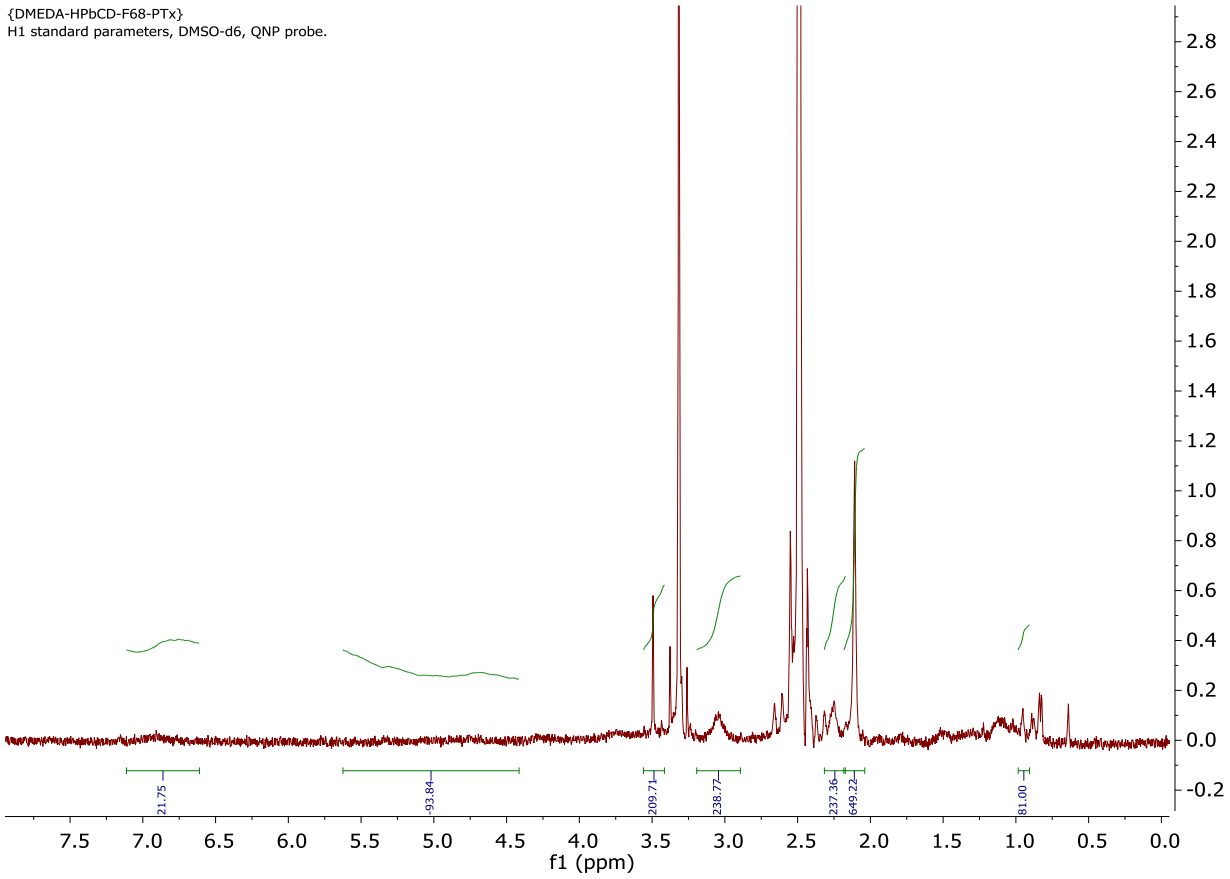

Figure S7: ^{1}H NMR Spectrum of DMEDA-HPCD:F68 Pluronic PR

^{1}H NMR Spectrum of DMEDA-HPCD:L35 Pluronic PR

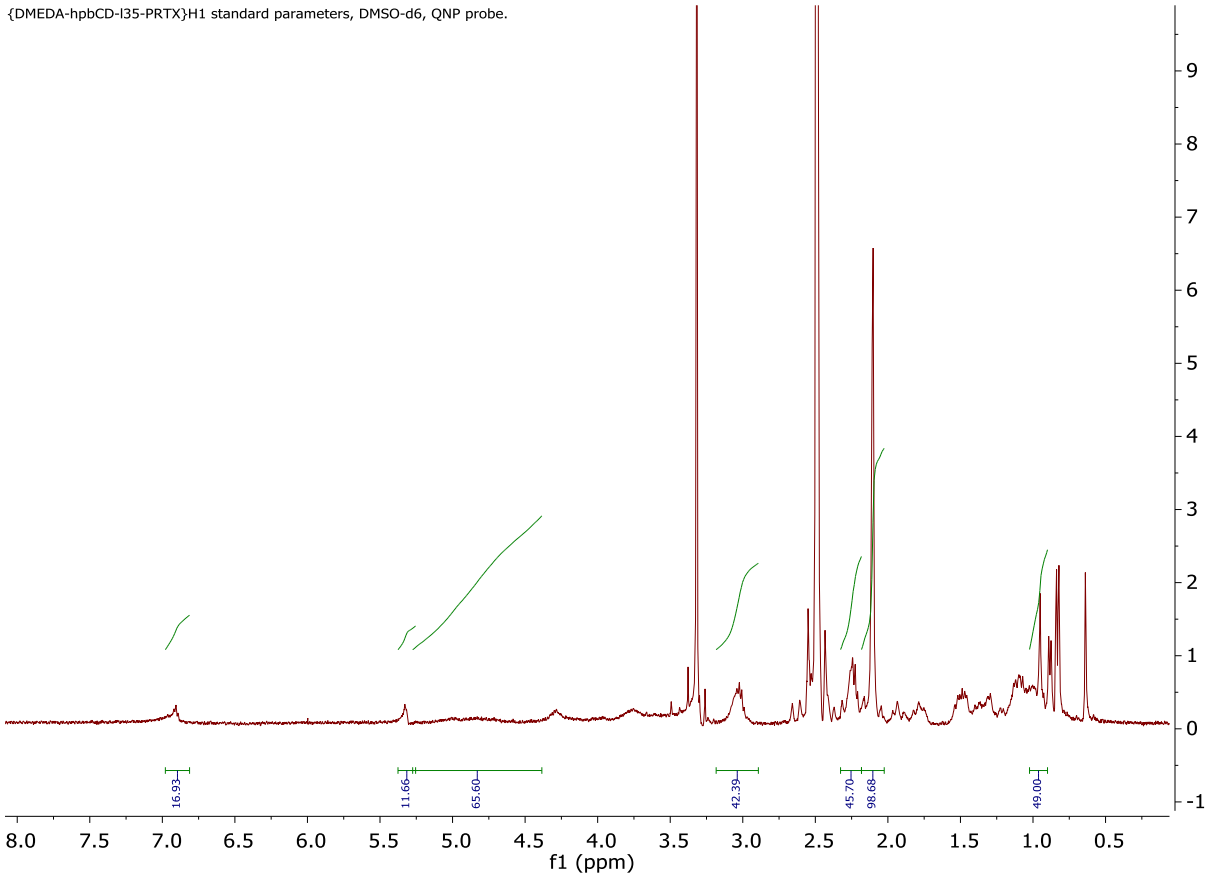

Figure S8: ^{1}H NMR Spectrum of DMEDA-HPCD:L35 Pluronic PR

^{1}H NMR Spectrum of DMEDA-HPCD:L64 Pluronic PR

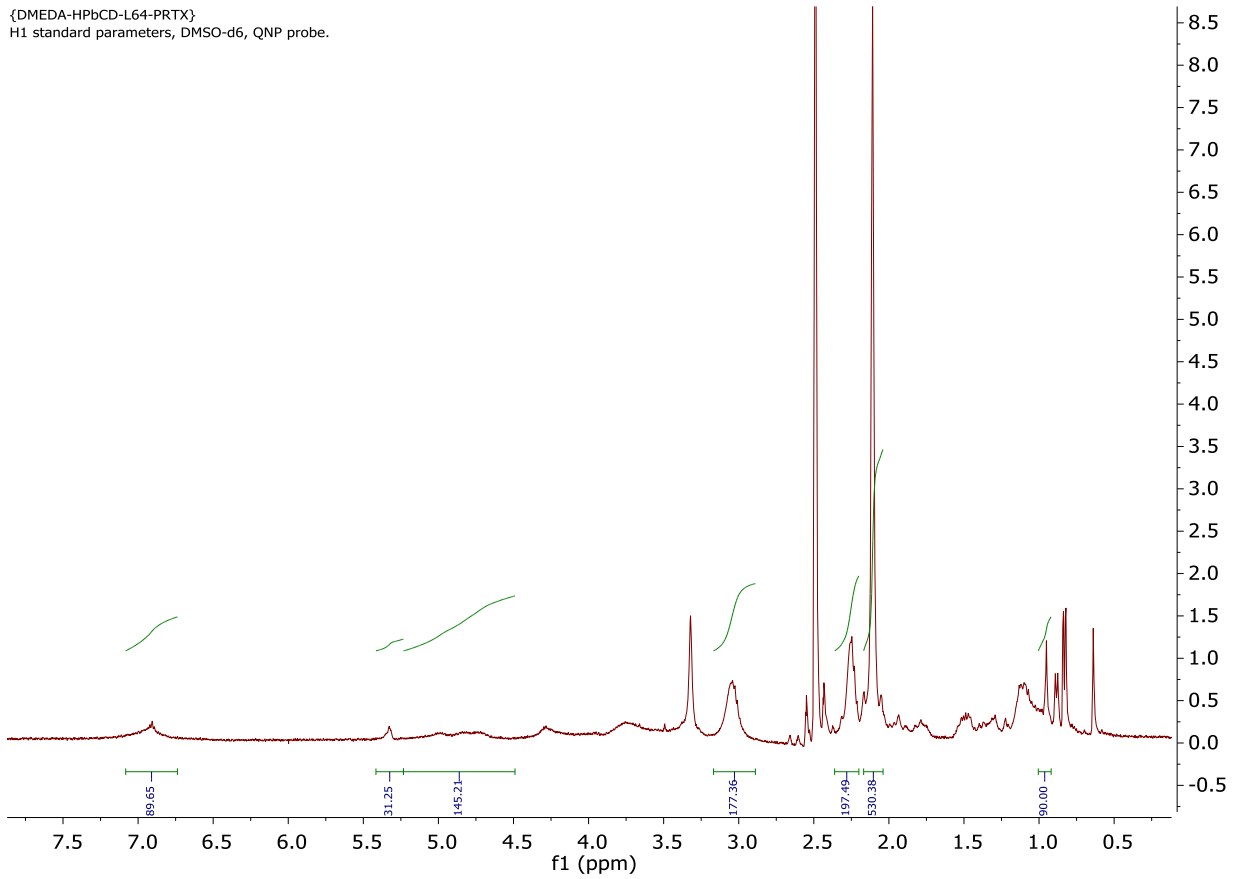

Figure S9: ^{1}H NMR Spectrum of DMEDA-HPCD:L64 Pluronic PR

^{1}H NMR Spectrum of DMEDA-HPCD:L81 Pluronic PR

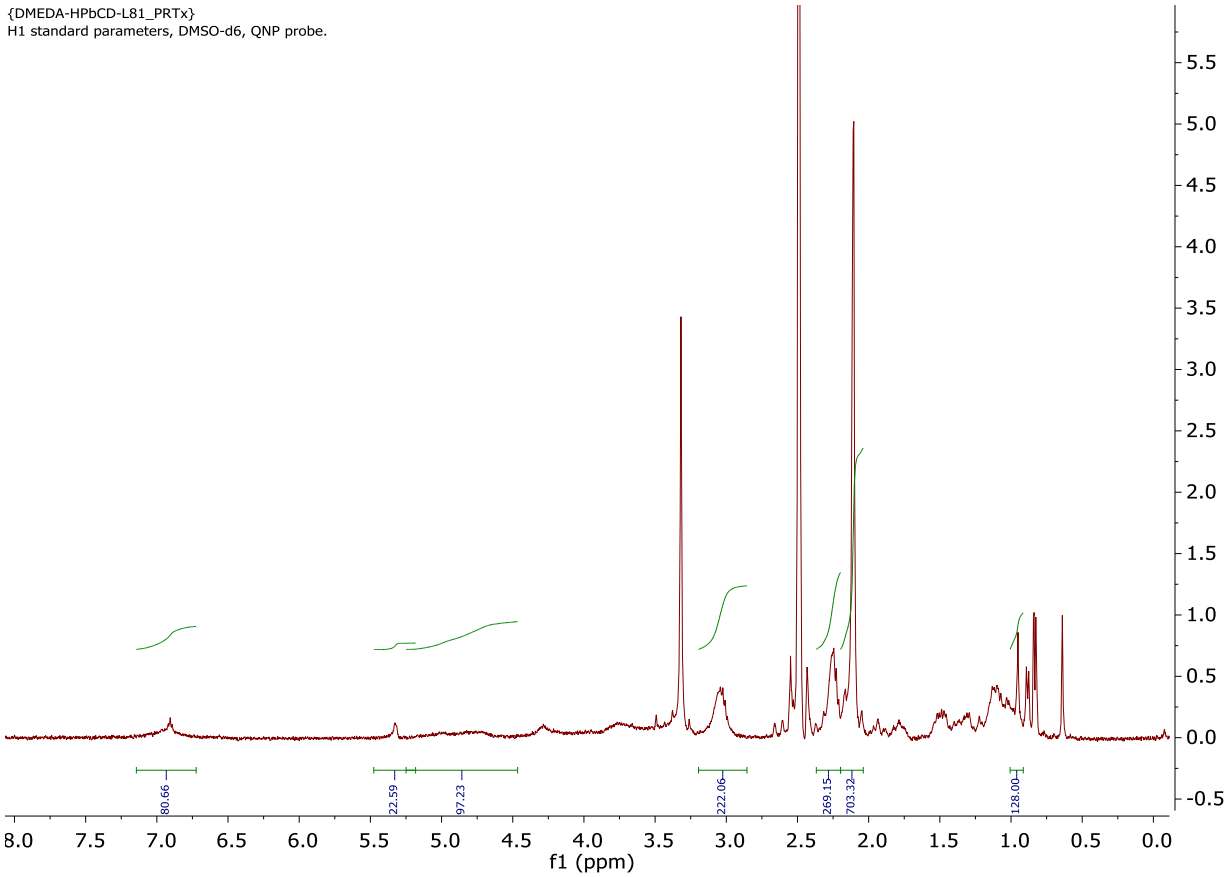

Figure S10: ^{1}H NMR Spectrum of DMEDA-HPCD:L81 Pluronic PR^{+}

2. AFM images of PR^+:pDNA complexes

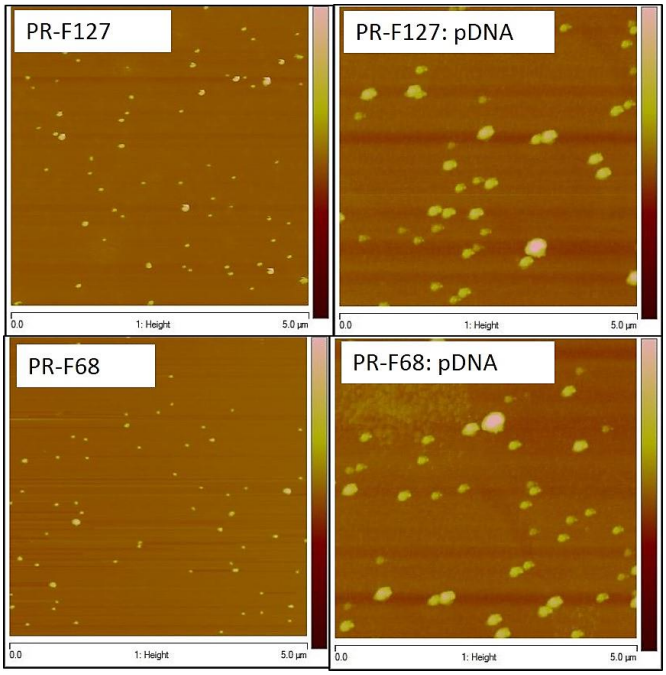

Figure S11: AFM images of PR^+ and PR^+:pDNA complexes at N/P ratio of 30.

3. Gel shift assay of PR^+:pDNA complexes

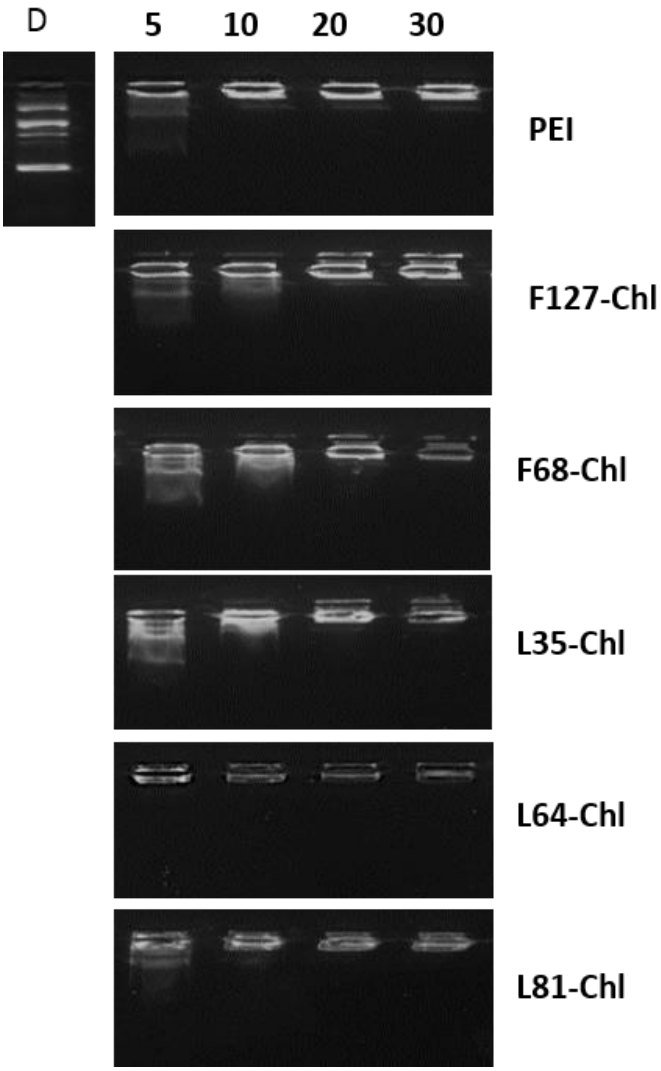

Figure S12. Gel retardation assay showing complexation ability of bPEI, F127-, F68-, L35-, L64- and L81-based PR^+ complexes formed at different N/P ratios.

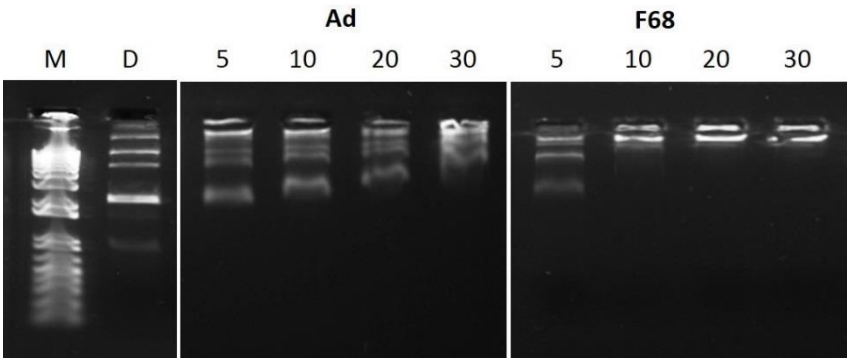

Figure S13. Gel retardation assay showing comparison between complexation ability of Admantane-PVA-PEG pendant polymer system with that of F68-based PR^+ at different N/P ratios.

4. *PicoGreen assay of PR^+:pDNA complexes*

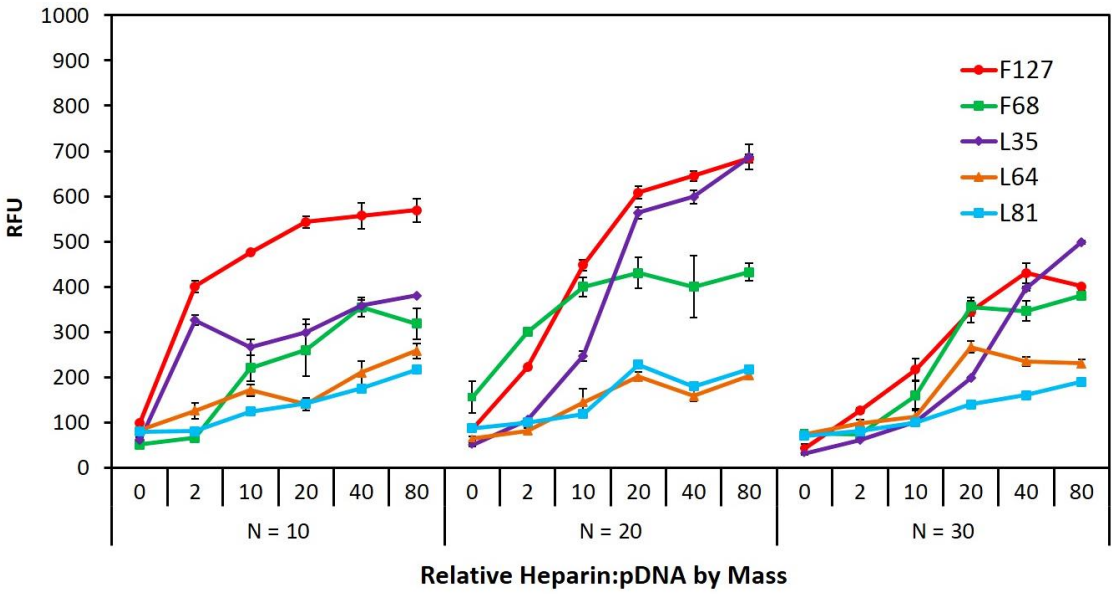

Figure S14. PicoGreen competitive binding assay showing colloidal stability of F68-, L35-, L64- and L81- based PR^+:pDNA complexes formulated at different N/P ratios. All the complexes were assayed for pDNA binding affinity at increasing heparin concentration from 0 to 80 weight ratio relative to pDNA.

5. *Raw flow-cytometry data for cellular uptake, transfection and cell viability using SYTOX AADvanced dead stain (red)*

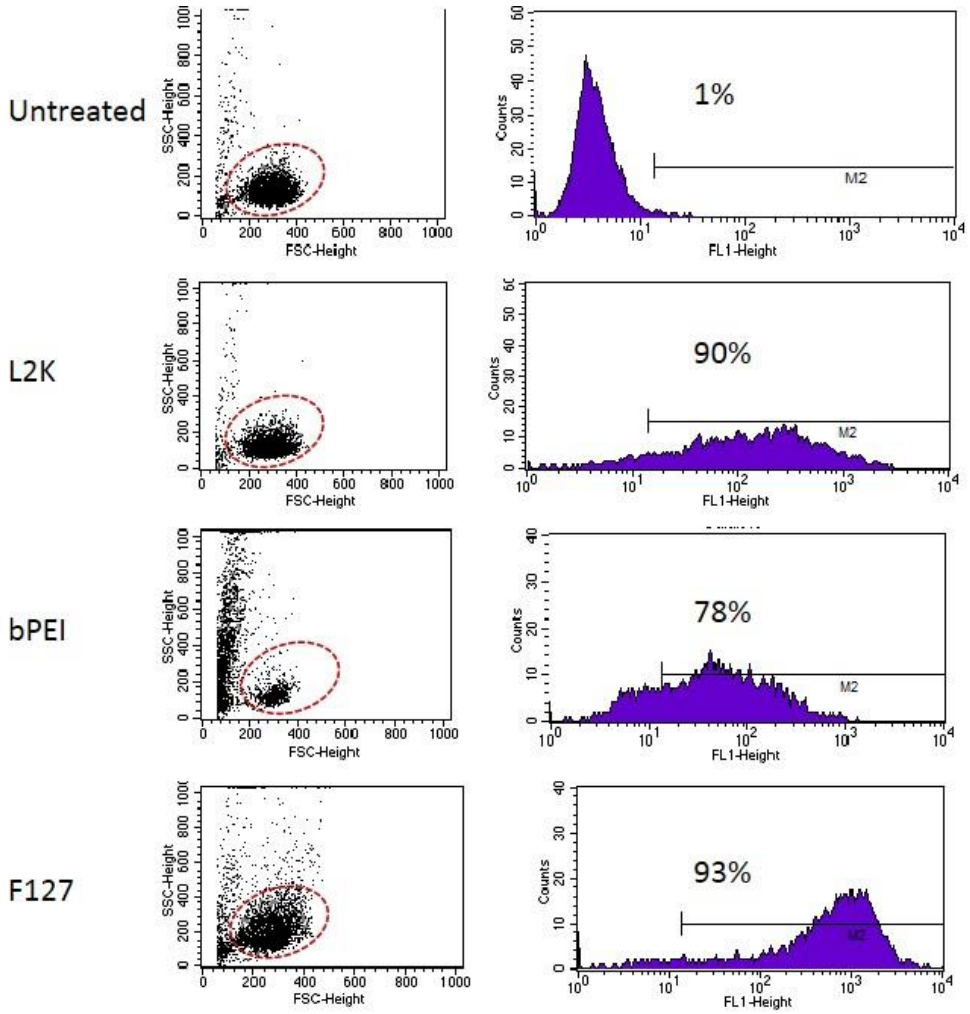

Figure S15. Representative FACS dot and histogram plots of cellular uptake of untreated, L2K, bPEI, and F127-based PR^+ complexes in NIH 3T3 cells. bPEI, and F127-based PR^+:pDNA complexes were formulated at N/P = 30 before addition to HeLa cells. Complexes of 1µg FITC-labeled pDNA were incubated with the cells in 10% serum-supplemented media for 4 h and analyzed. A total population of 10,000 cells were analyzed in each case as described in Experimental Methods.

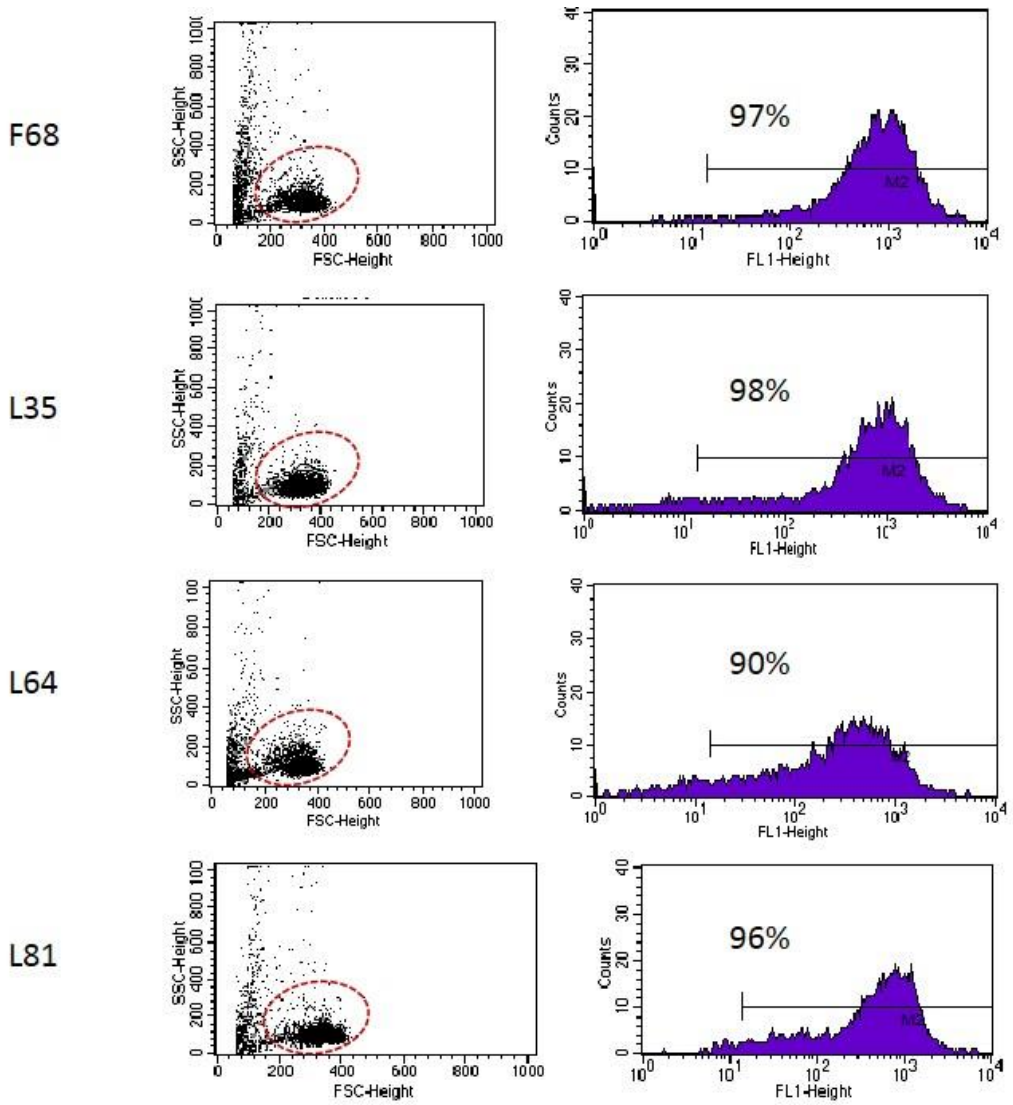

Figure S16. Representative FACS dot and histogram plots of cellular uptake of F68-, L35-, L64- and L81- based PR^{+}:pDNA complexes formulated at N/P = 30 prior to treatment of NIH 3T3 cells. Complexes containing 1µg FITC-labeled pDNA were incubated with the cells in 10% serum-supplemented media for 4 h and analyzed in each case as described in Experimental Methods.

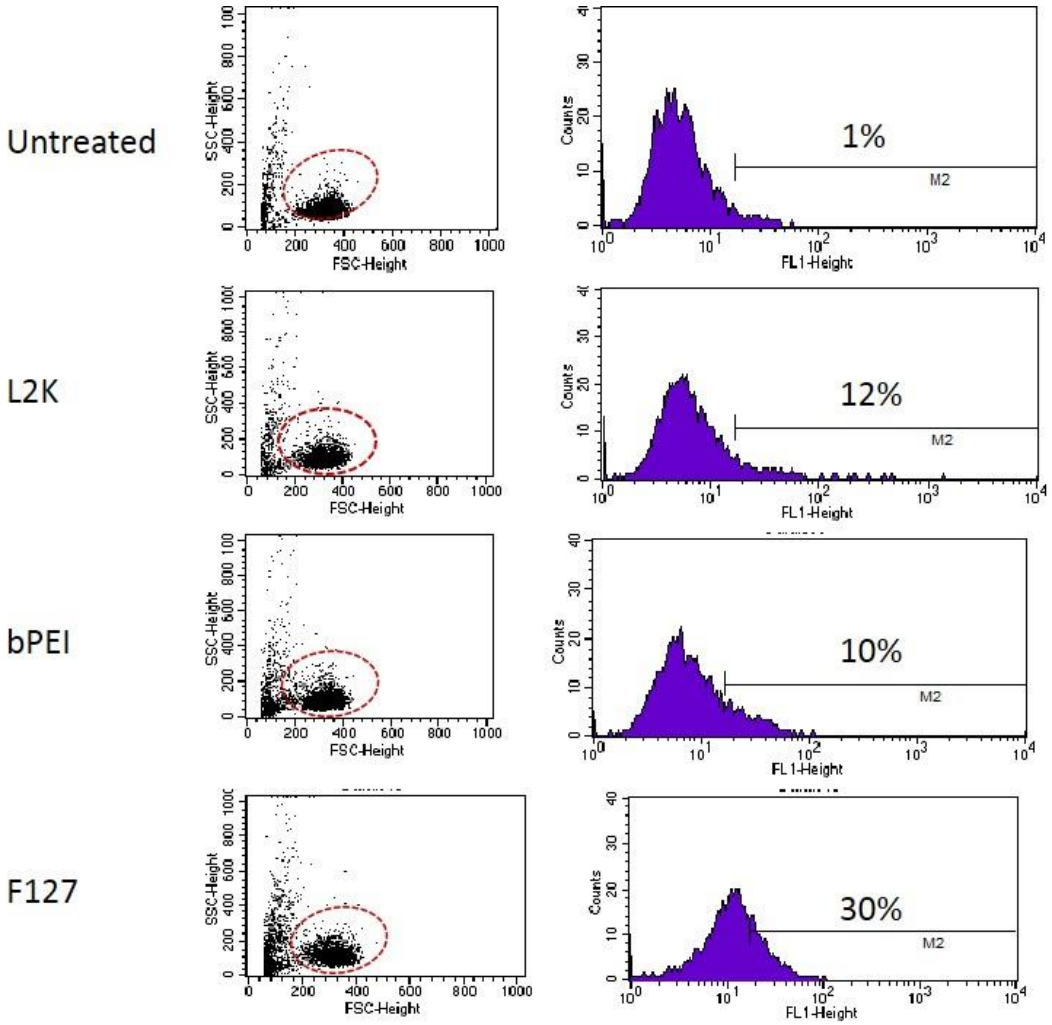

Figure S17. Representative FACS dot and histogram plots of untreated and pDNA transfected NIH 3T3 cells using L2K, bPEI, and F127-based PR^{+}. bPEI and F127-based PR^{+} complexes were formulated at N/P = 30. Complexes of 1μg pDNA were incubated with the cells in 10% serum-supplemented media for 4 h and analyzed after a further 36 h incubation as described in Experimental Methods.

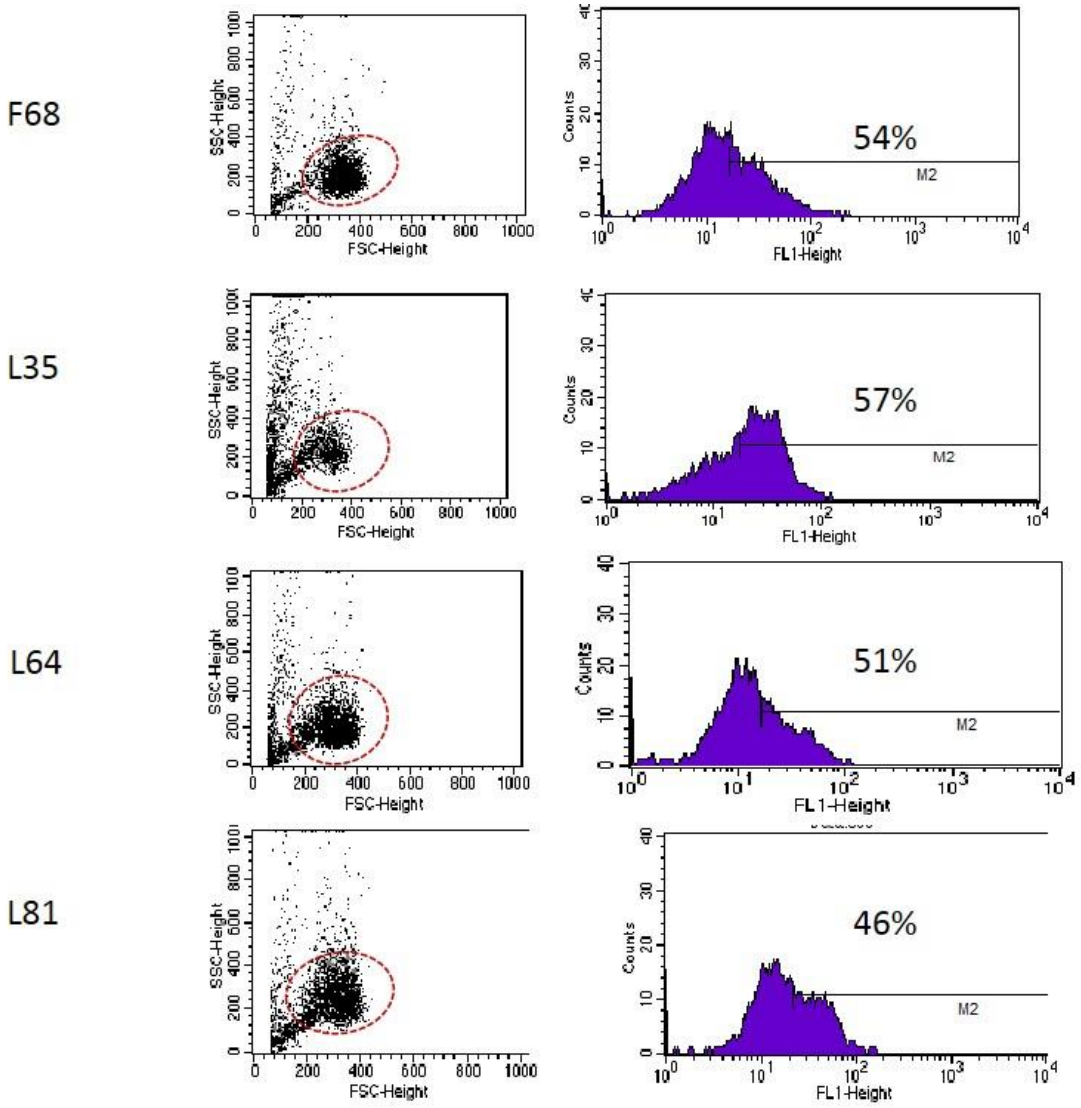

Figure S18. Representative FACS dot and histogram plots of pDNA transfection by F68-, L35-, L64- and L81-based PR^{+}:pDNA complexes formulated at N/P = 30 in NIH 3T3 cells. Complexes of 1μg FITC-labeled pDNA were incubated with the cells in 10% serum-supplemented media for 4h and analyzed after a further 36 h incubation as described in Experimental Methods.

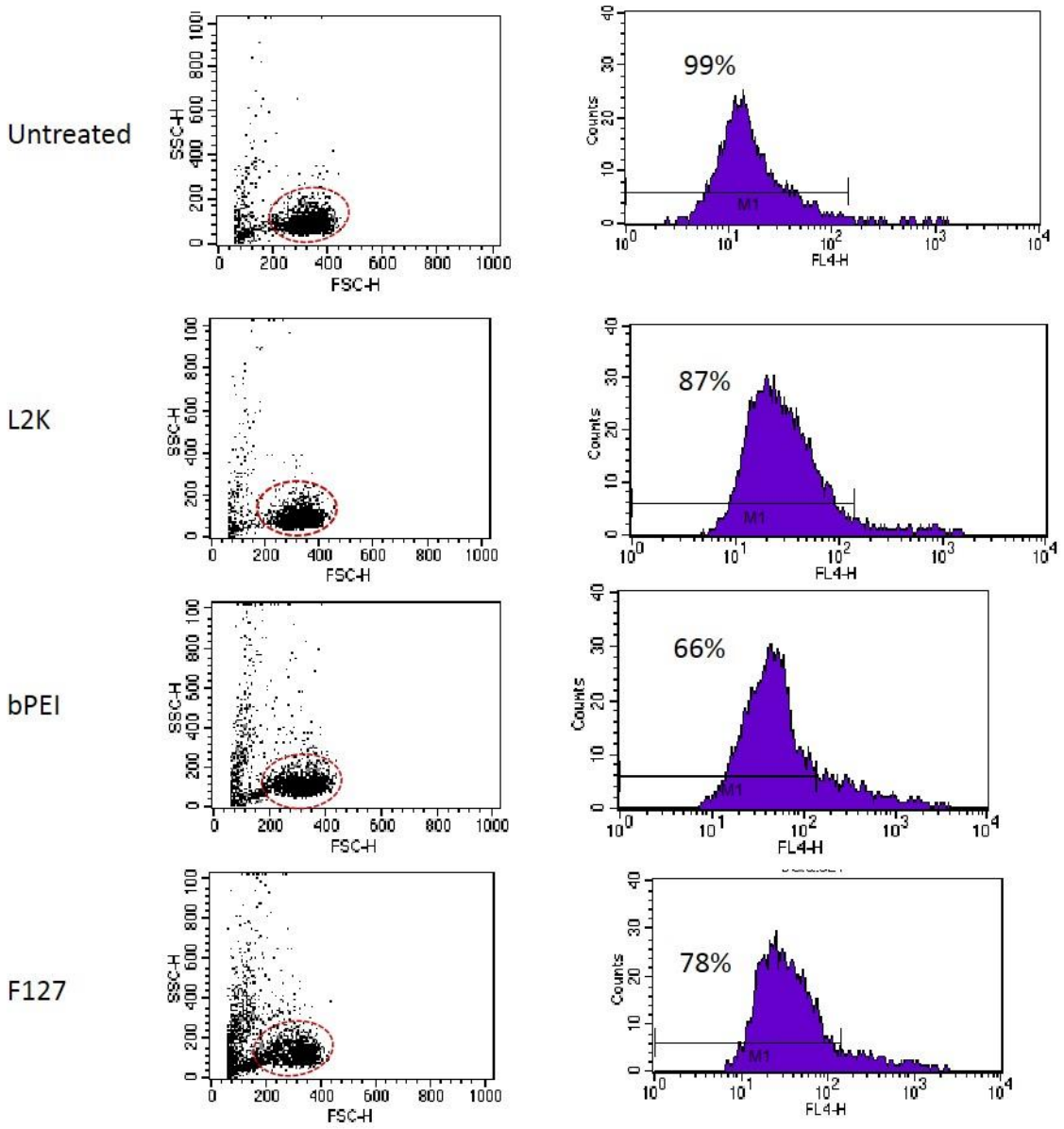

Figure S19. Representative FACS dot and histogram plots of SYTOX AADvanced labeling of NIH 3T3 cells treated with L2K, bPEI, and F127-based PR^+:pDNA complexes. bPEI and F127-based PR^+ complexes were formulated at N/P = 30. Complexes of 1µg AcGFP1 pDNA were incubated with the cells in 10% serum-supplemented media for 4 h and analyzed after a further 36 h incubation as described in Experimental Methods.

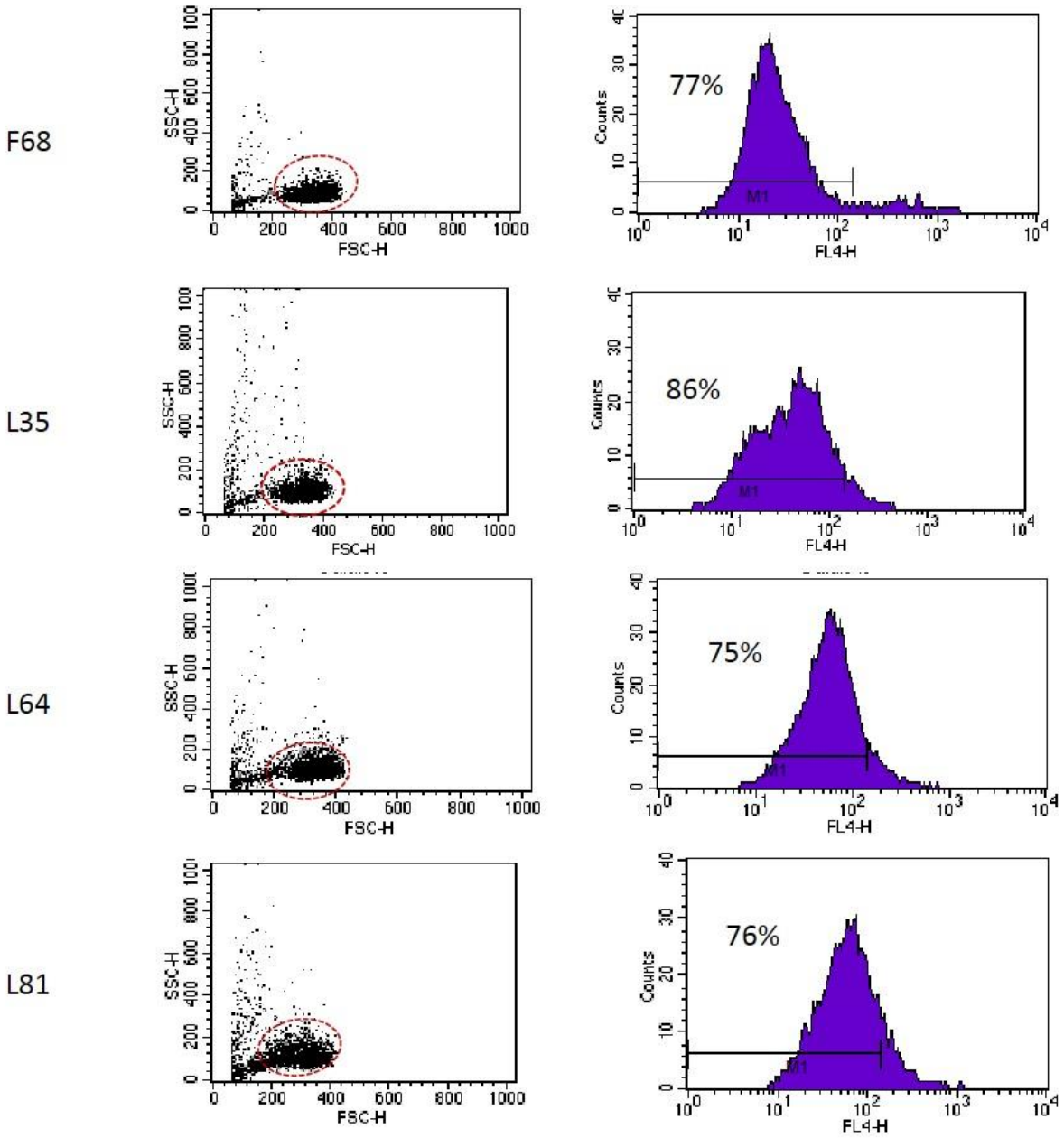

Figure S20. Representative FACS dot and histogram plots of SYTOX AADvanced dead stain (red) labeling of NIH 3T3 cells treated with F68-, L35-, L64- and L81-based PR^{+}:pDNA complexes formulated at N/P = 30. Complexes of 1µg pDNA were incubated with the cells in 10% serum-supplemented media for 4 h and analyzed after a further 36 h incubation as described in Experimental Methods.