SUPPLEMENTARY MATERIAL

Nebulization of siRNA for inhalation therapy based on a microfluidic surface acoustic wave platform

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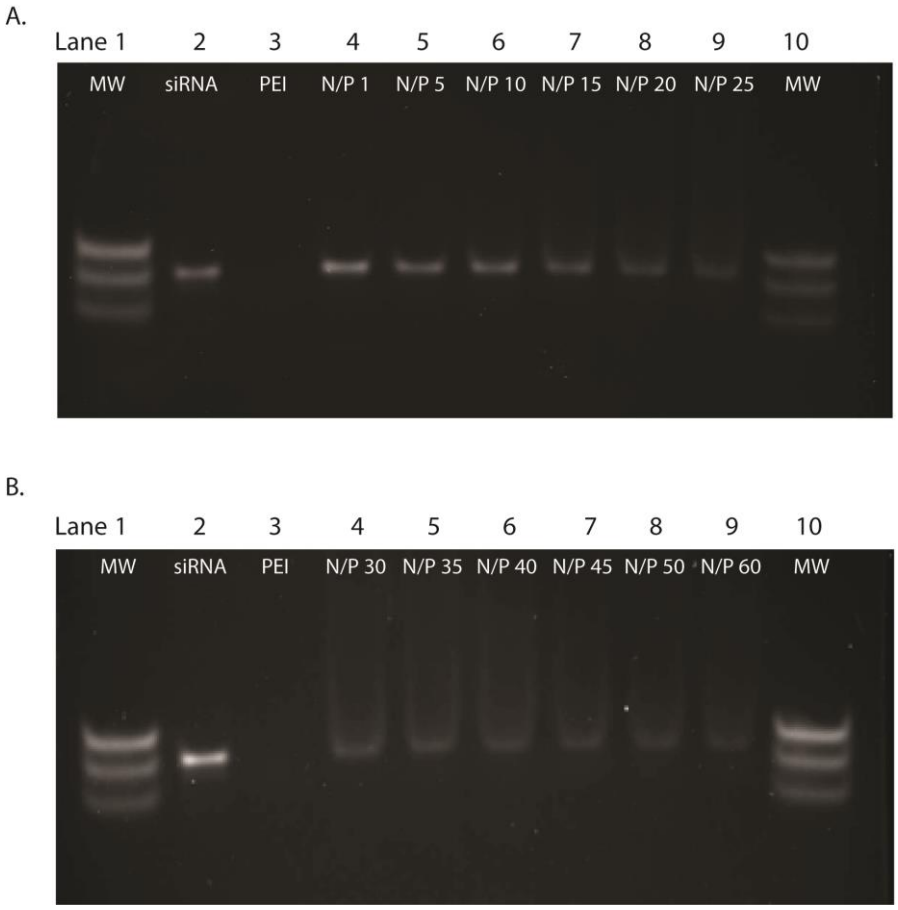

Figure S1. Non-denaturing polyacrylamide gel electrophoresis showing the complexation of siRNA with linear PEI at N to P ratios of (A.) 1, 5, 10, 20, 25; and (B.) 30, 35, 40, 45, 50, 60. The bands representing a siRNA molecular weight ladder (17, 21, 25 bp), naked siRNA and PEI alone are also shown in both gels.

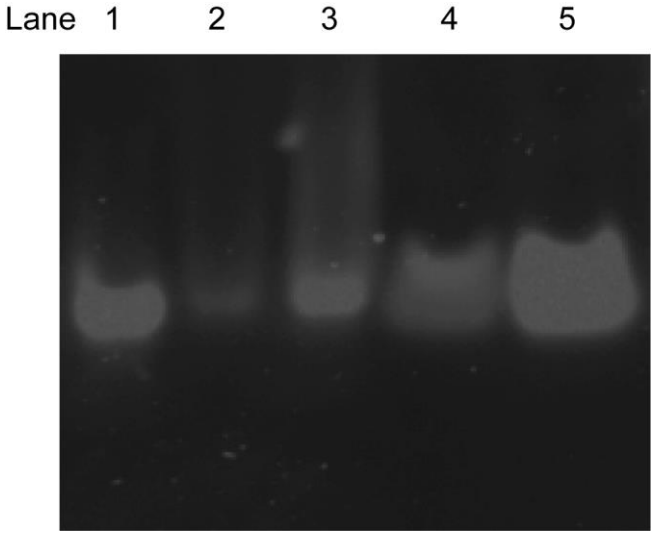

Figure S2. Non-denaturing polyacrylamide gel electrophoresis showing the release of siRNA from the complexes by incubation with heparin. Lane 1: Naked siRNA; Lane 2: siRNA/PEI complexes at N/P 30 (in sodium acetate, Ph 5.2); Lane 3: siRNA/PEI complexes at N/P 30 (in water); Lane 4: siRNA/PEI complexes at N/P 30 in the presence of heparin (in sodium acetate, Ph 5.2); and Lane 5: siRNA/PEI complexes at N/P 30 in the presence of heparin (in water).

Table S1. Hydrodynamic diameters of INTERFERin/siRNA complexes

Complex[a]	Diameter[b] (nm)
INTERFERin/siRNA = 1	620 +/- 80
INTERFERin/siRNA = 2	545 +/- 220
INTERFERin/siRNA = 3	455 +/- 110

[a] INTERFERin/siRNA of 1 refers to the suggested ratio as per the manufacturer's protocol. INTERFERin/siRNA of 2 and 3 refer to 2x and 3x the recommended volume of INTERFERin, respectively.

[b] Measured using dynamic light scattering, in 25mM sodium acetate, pH 5.2

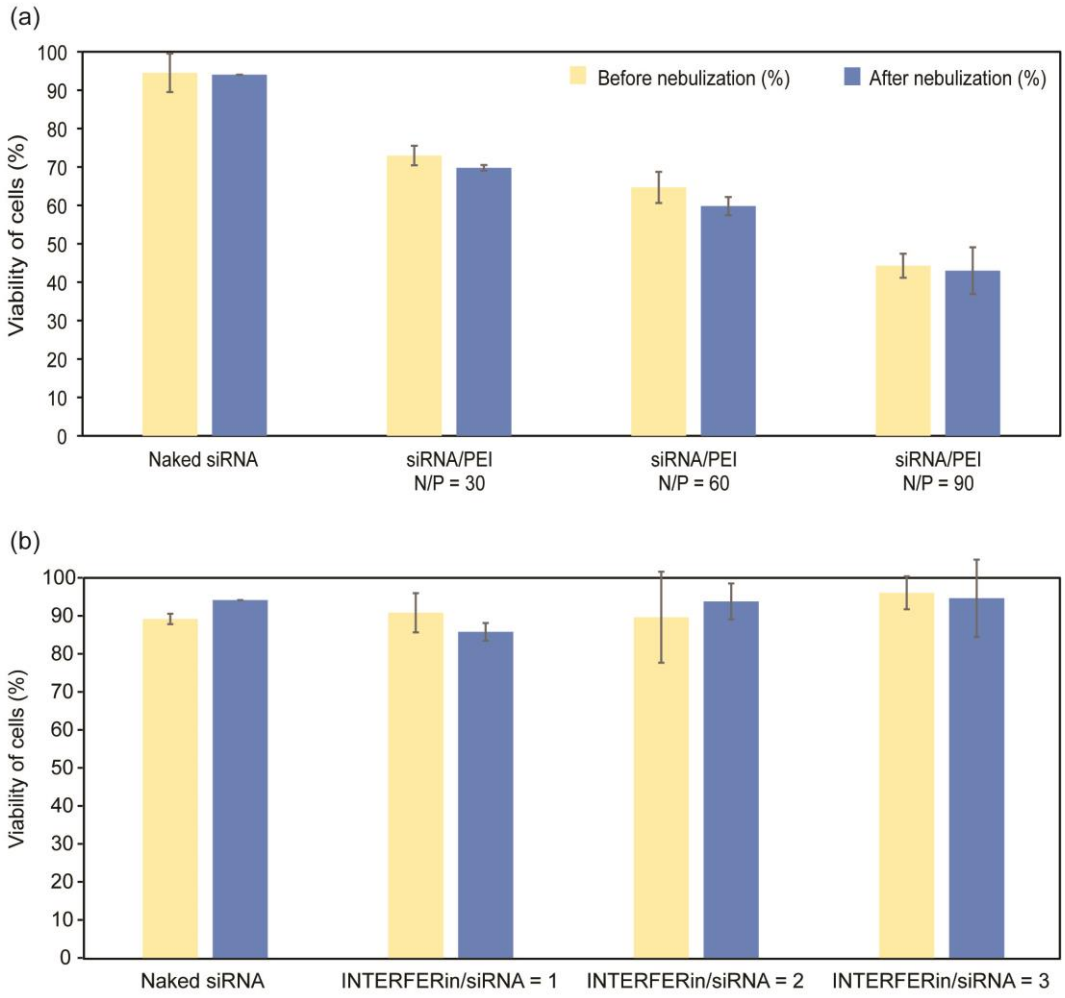

Figure S3. Viability of cells treated with naked siRNA and siRNA complexed with cationic PEI (a) or INTERFERin (b) at various ratios. Cells were incubated with nebulized or non-nebulized siRNA samples and incubated for 48 h at 37°C with 5% CO_2 prior to measurement of cell viability using the Alamar blue reagent. The viability of treated samples has been normalized against the viability of untreated cells.