Supplementary Information

Mechanistic Insight into Receptor-mediated Delivery of Cationic-β-Cyclodextrin:Hyaluronic Acid-Adamantamethamidyl Host:Guest pDNA Nanoparticles to $CD44^+$ Cells

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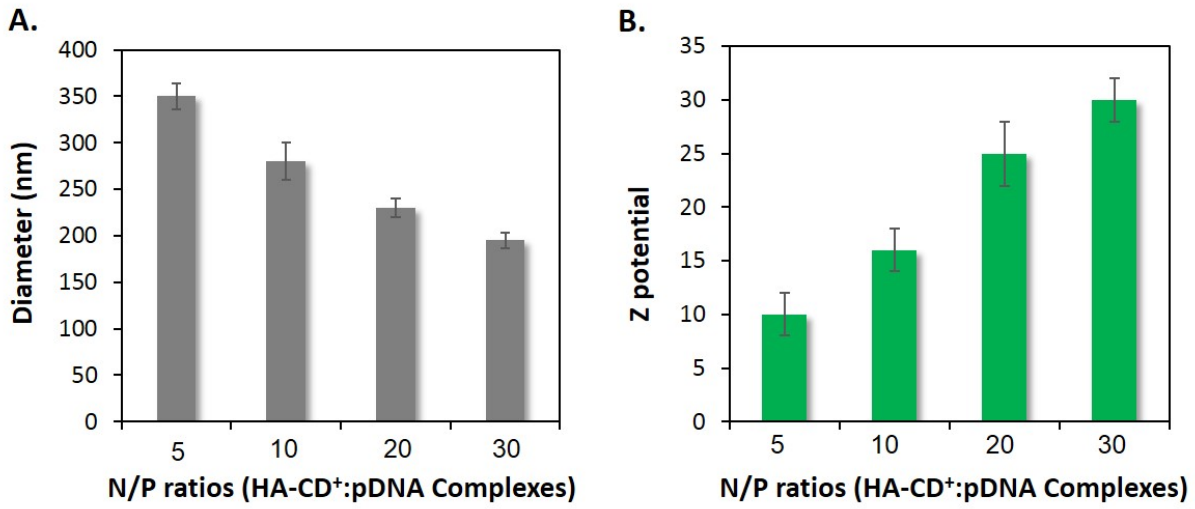

Figure S1. Physical Characterization of HA-Ad:CD-PEI$^+$:pDNA Complexes: Size and ζ-Potential as a Function of N/P ratio. (A) Particle diameter, determined by dynamic light scattering (DLS) analysis, as a function of N/P; **(B)** ζ-potentials of HA-Ad:CD-PEI$^+$:pDNA complexes formulated at various N/P ratios. For DLS and ζ-potential analysis, HA-Ad:CD-PEI$^+$:pDNA particles were formulated and measured using Zetasizer Nano-S, (Malvern Instruments Ltd.) at 20 °C with a scattering angle of 90°. At least 40 measurements were made and averaged for each sample for **ζ-**potential as well as size determination.

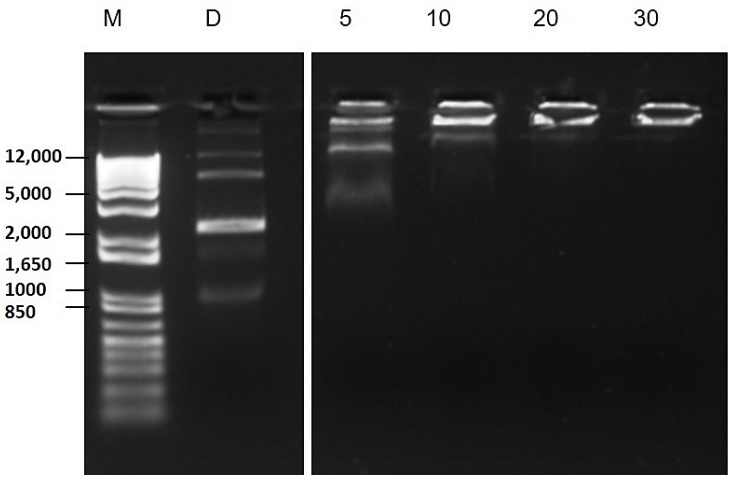

Figure S2. Colloidal Stability of HA-Ad:CD-PEI^{+}:pDNA as a Function of N/P ratio.

Gel retardation assay showing HA-CD-PEI^{+}:pDNA complex stability at different N/P ratios. M-standard pDNA ladder, D-free pDNA, 5, 10, 20 & 30 represents the N/P ratios at which HA-Ad:CD-PEI^{+}:pDNA are formulated. Free plasmid (D) showing various forms of plasmid DNA typically observed in gel electrophoresis[1]. In general, pDNA exist in various forms such as nicked (relaxed circular plasmid), supercoiled form and topoisomeric forms. Most commonly, the most relaxed form of pDNA is observed to migrated the least (in this case bands >5000), supercoiled the most (in this case bands <1650), while all other intermediate forms in the middle range (1650 to 5000).

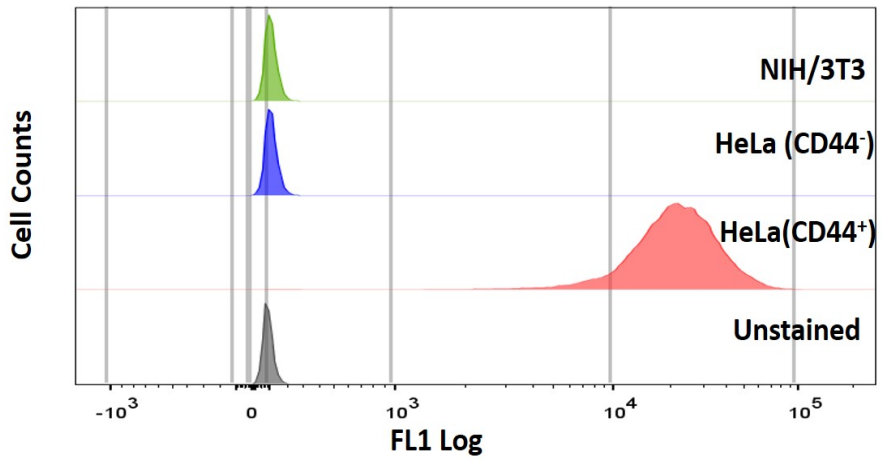

Figure S3. Flow cytometry data for CD44 analysis using Brilliant Violet 421® antihuman CD44 antibody. (processed through FlowJo v7. software)

CD44 Expression Knockdown in HeLa Cells. Expression of $CD44^+$ receptors was knocked down using HCAM siRNA (h) in HeLa cells. HCAM siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knockdown CD44 expression. $CD44^+$ HeLa cells were incubated at 37 °C in a CO_2 incubator for 24 h to achieve 70-80% confluency. siRNA (75 pmol), dissolved in 50 µl of nanopure water, was mixed with 3 µL Lipofectamine 2000 (dissolved in 50 µL of water). The mixture was allowed to incubate for 30 min to form transfection complexes. The resulting complexes were dissolved in DMEM (serum free) media, followed by incubation with cells for 6 h. After incubation, the medium was aspirated and replaced with fresh DMEM media containing 5% serum. The cells were allowed to grow for an additional 48 h before analysis.

Analysis of CD44 Expression in HeLa Cells. $CD44^+$ HeLa, $CD44^-$ HeLa, and NIH 3T3 cells were used to study the expression of CD44 receptors by plating 75,000 cells per well in 24-well plates and incubating for 24 h before the experiment. Cells were stained with amto-CD44-BV421 (BD Biosciences) at a concentration of 1 µg/mL in DMEM media for 30 min. After incubation, the spent media was removed and the cells were washed 3 times with PBS before trypsinization. The cells were then collected and analyzed using anti-CD44-BV421 by flow cytometry using a BD FACS Aria III.

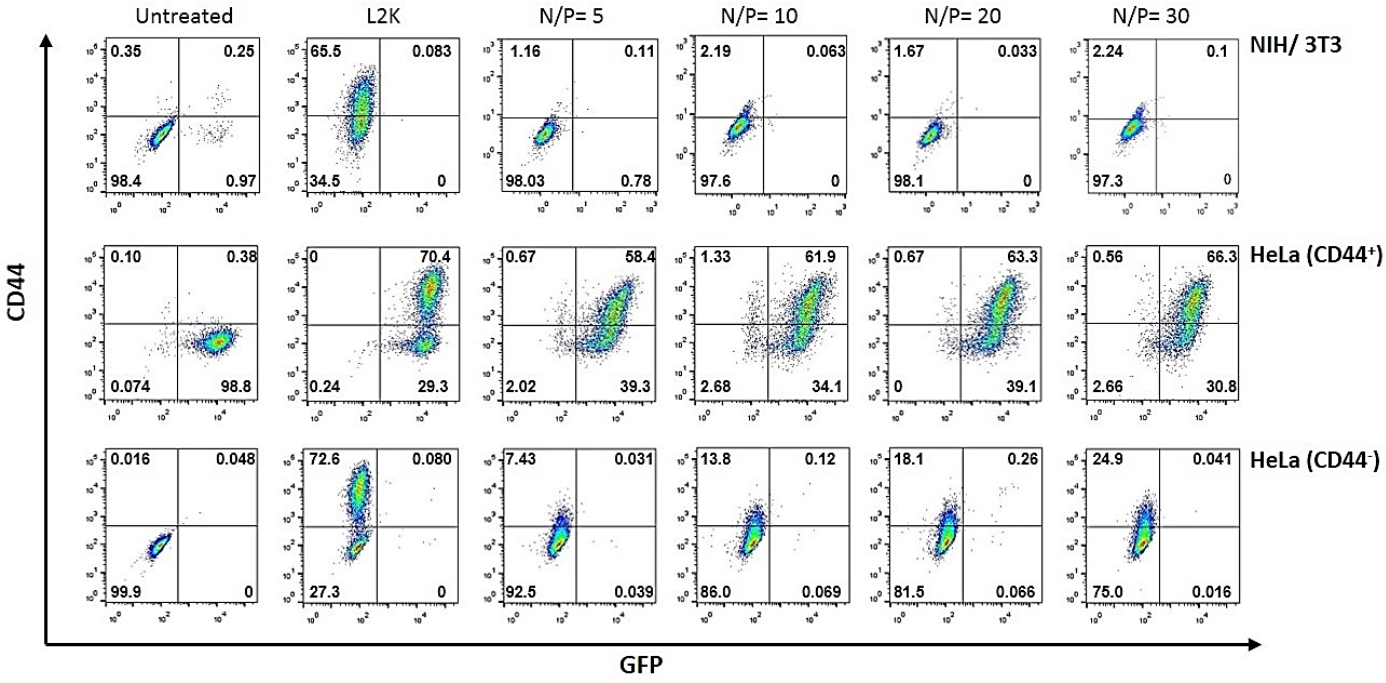

Figure S4. Flow cytometry data for cellular uptake of HA-Ad:CD-PEI^{+}:pDNA and L2K:pDNA complexes after 4h incubation with HeLa and NIH 3T3 cells.

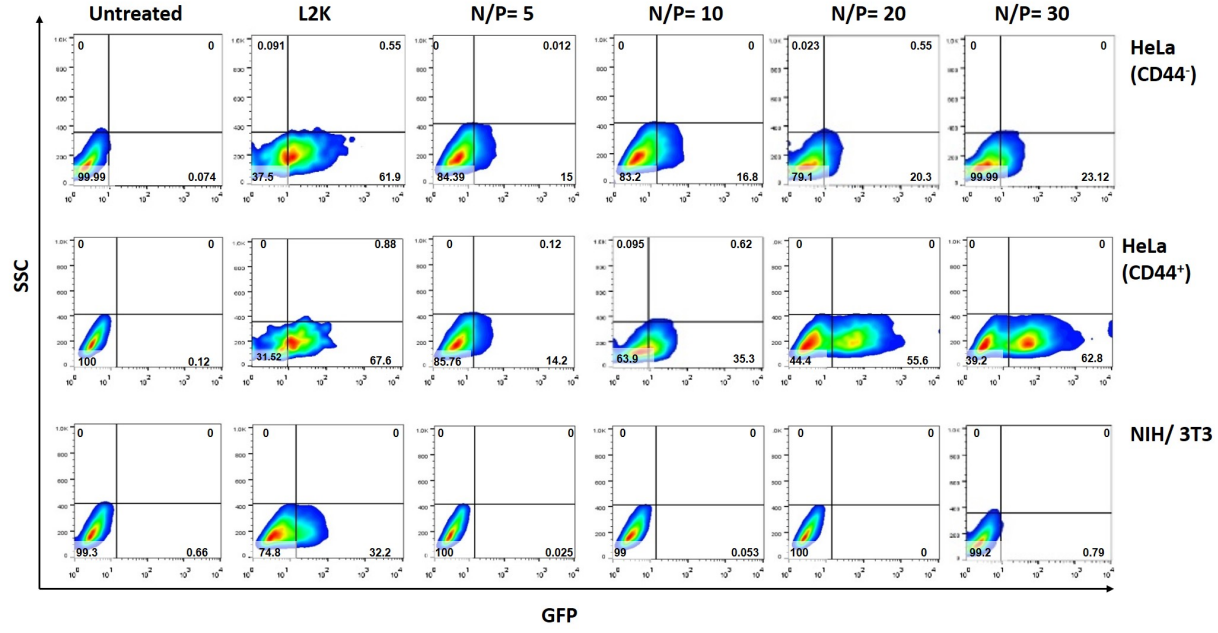

Figure S5. Flow cytometry data for transfection analysis of HA-Ad:CD-PEI^{+}:pDNA and L2K:pDNA complexes after 4 h incubation with HeLa and NIH 3T3 cells

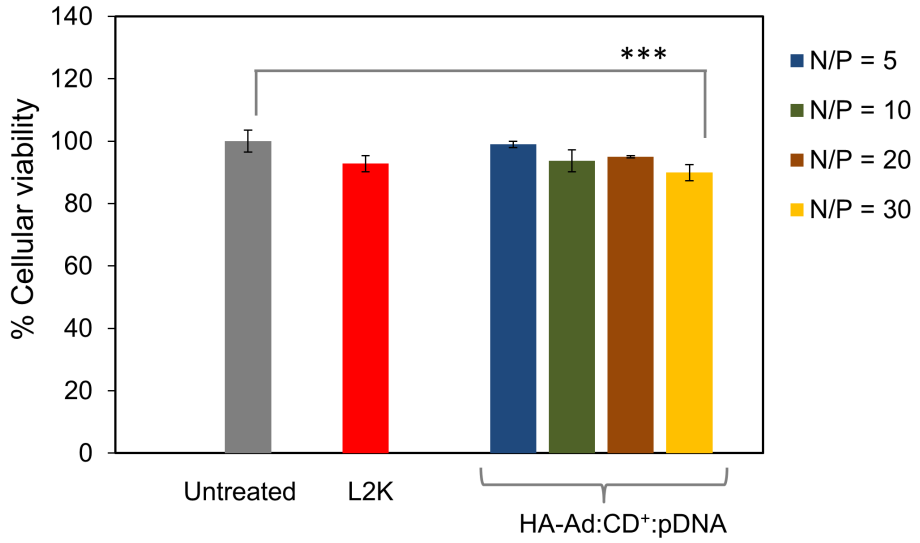

Figure S6. MTS cell viability assay for HeLa cells, after 24 h exposure to HA-Ad:CD-PEI^{+}:pDNA and L2K:pDNA complexes. For MTS assay, the cells at densities of 10,000 cell/well were seeded in 96-well microtiter plates and incubated with HA-Ad:CD-PEI^{+}:pDNA and L2K:pDNA complexes for 24 h. Following the incubation, MTS reagent was added to each sample and further incubated for 2 h and then absorbance of each well was measured using a plate reader at a wavelength of 490 nm.

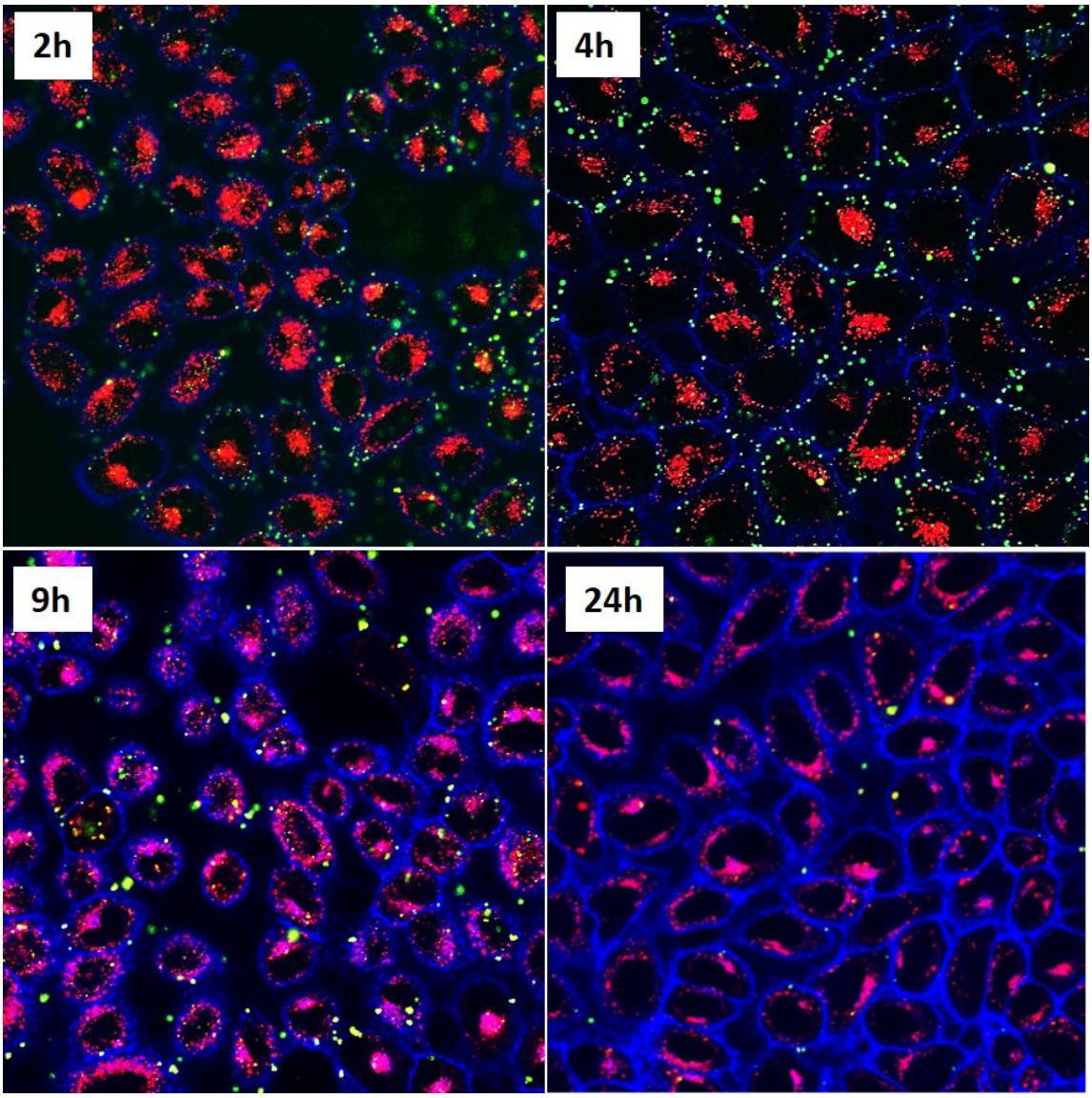

Figure S7. Confocal microscopy images of HA-Ad:CD-PEI^{+}:pDNA complexes at N/P = 20; incubated at indicated time in h. Green: HA-Ad:CD-PEI^{+}:pDNA-FITC, Blue: Plasma membrane labeled with Alexa 689-WGA; Red: Lysosomes labeled with Lysotracker DND-99.

References:

1. Levene, S. D. Analysis of DNA Topoisomers, Knots, and Catenanes by Agarose Gel Electrophoresis DNA Topoisomerases: Methods and Protocols 2009, 582, 11-25