Supplementary file 1

Saponin conjugation improves p-CRISPR delivery by polyethylenimine modified carbon dots

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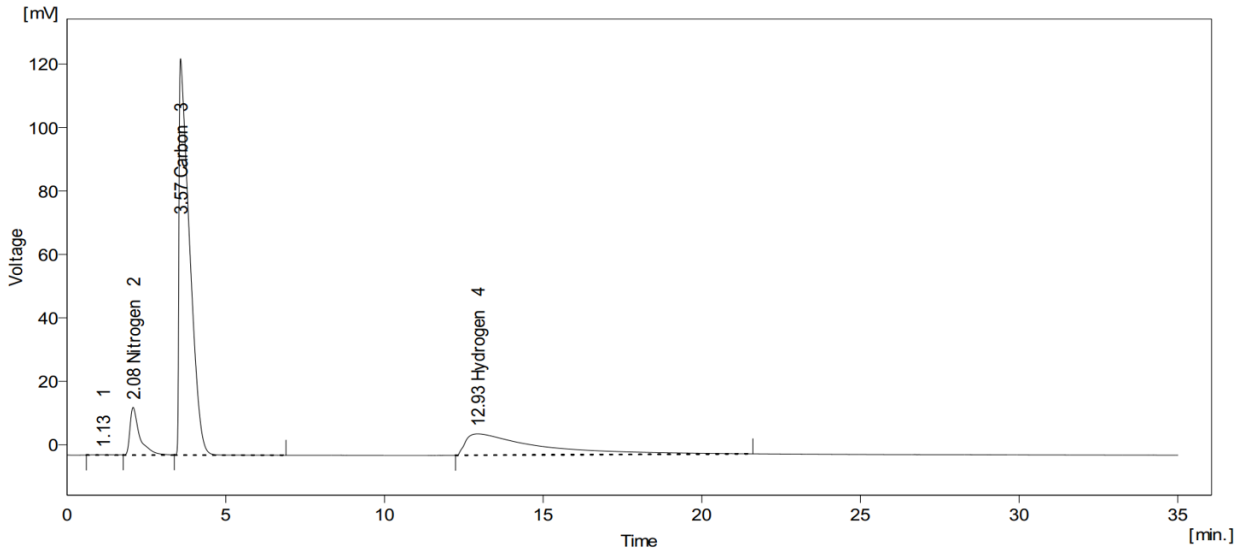


Fig S1: Elemental composition analysis of CD-PEI-Sap nanoparticles. This analysis confirmed the presence of carbon (30.86%), nitrogen (8.97%) and hydrogen (5.80%). The detectable nitrogen content originates from surface functionalization of the carbon dots with PEI $_{1.8kDa}$ and saponin, and contributes to the positive surface charge of the nanoparticles, which explains the improved plasmid binding and enhanced transfection efficiency.

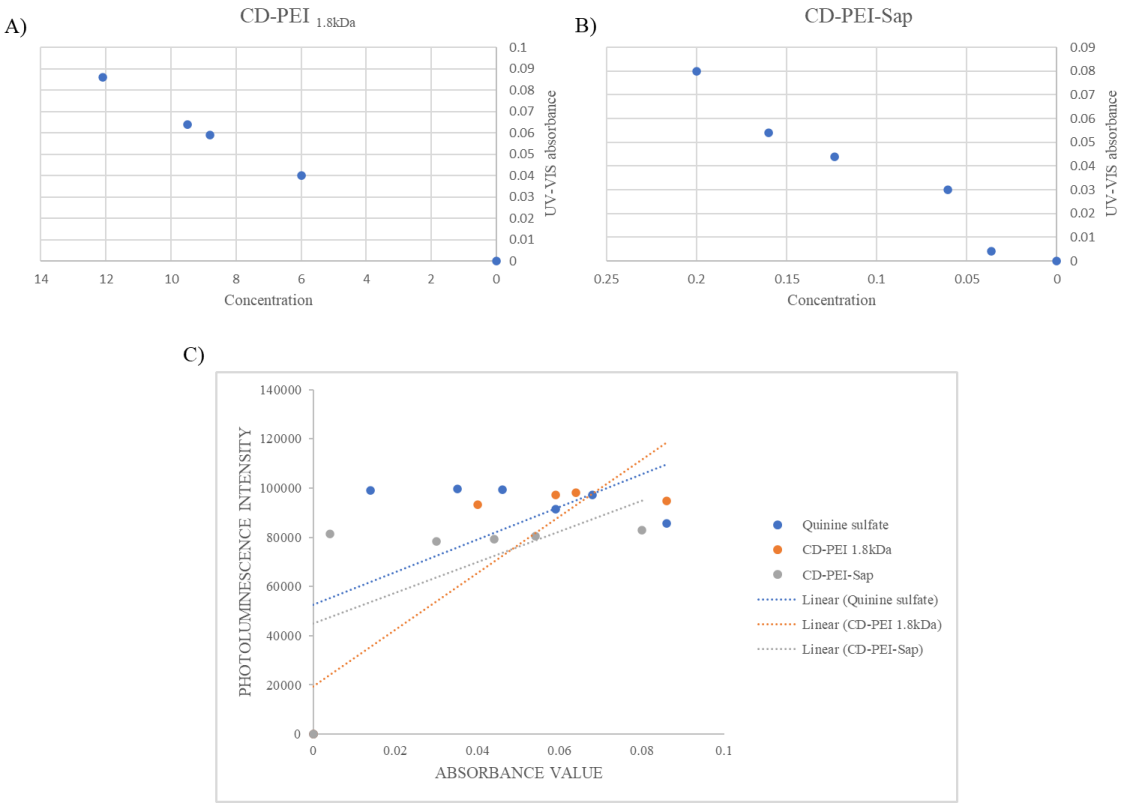
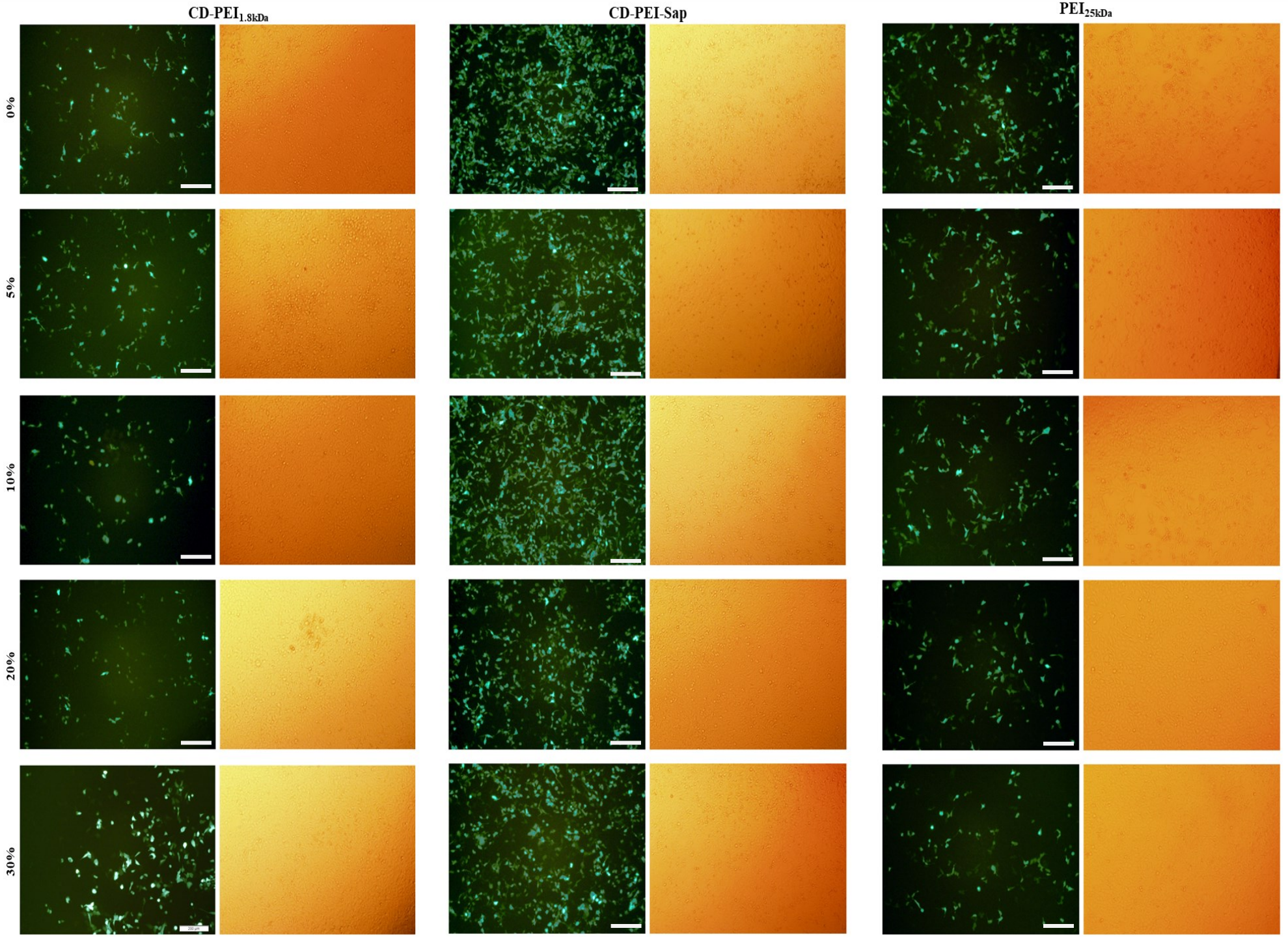
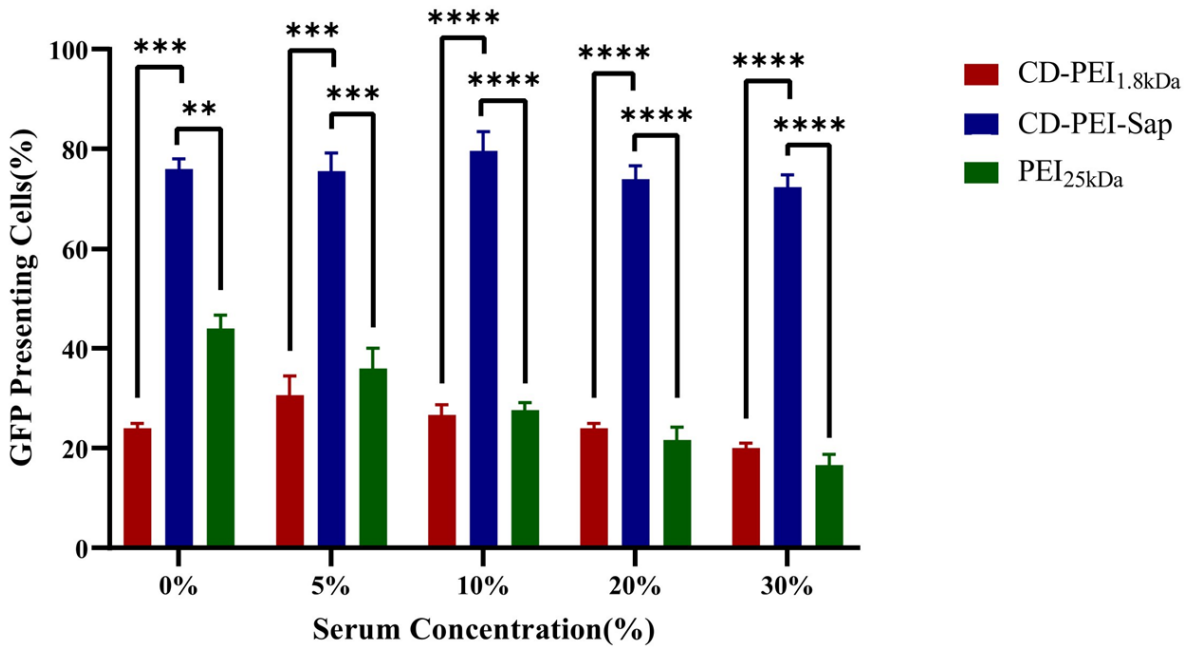


Fig S2: **Optical characterization and photoluminescence spectroscopy of carbon dot–based nanoparticles** A, B) UV-Visible spectra of CD-PEI $_{1.8kDa}$ and CD-PEI-Sap is provided in various scatterplots because concentration scales were different. C) The linear graph compares the integrated photoluminescence intensity (vertical axis) and the absorbance value (horizontal axis) of CD-PEI 1.8kDa, CD-PEI-Sap with a reference (quinine sulfate). PL spectra were recorded over a range of emission wavelengths using a fixed excitation wavelength of 340 nm. Quinine sulfate was used as a reference standard for comparison. The CD–PEI–Sap nanoparticles exhibited slightly lower PL intensity compared to CD–PEI $_{1.8\ kDa}$, which is attributed to fluorescence quenching effects induced by saponin incorporation in the carbon dot structure.

A)



B)


GFP Presenting Cells(%)
100
80
60
40
20
0
***
**
***
***
****
****
****
****
****
****
0%
5%
10%
20%
30%
Serum Concentration(%)
CD-PEI1.8kDa
CD-PEI-Sap
PEI25kDa

C)

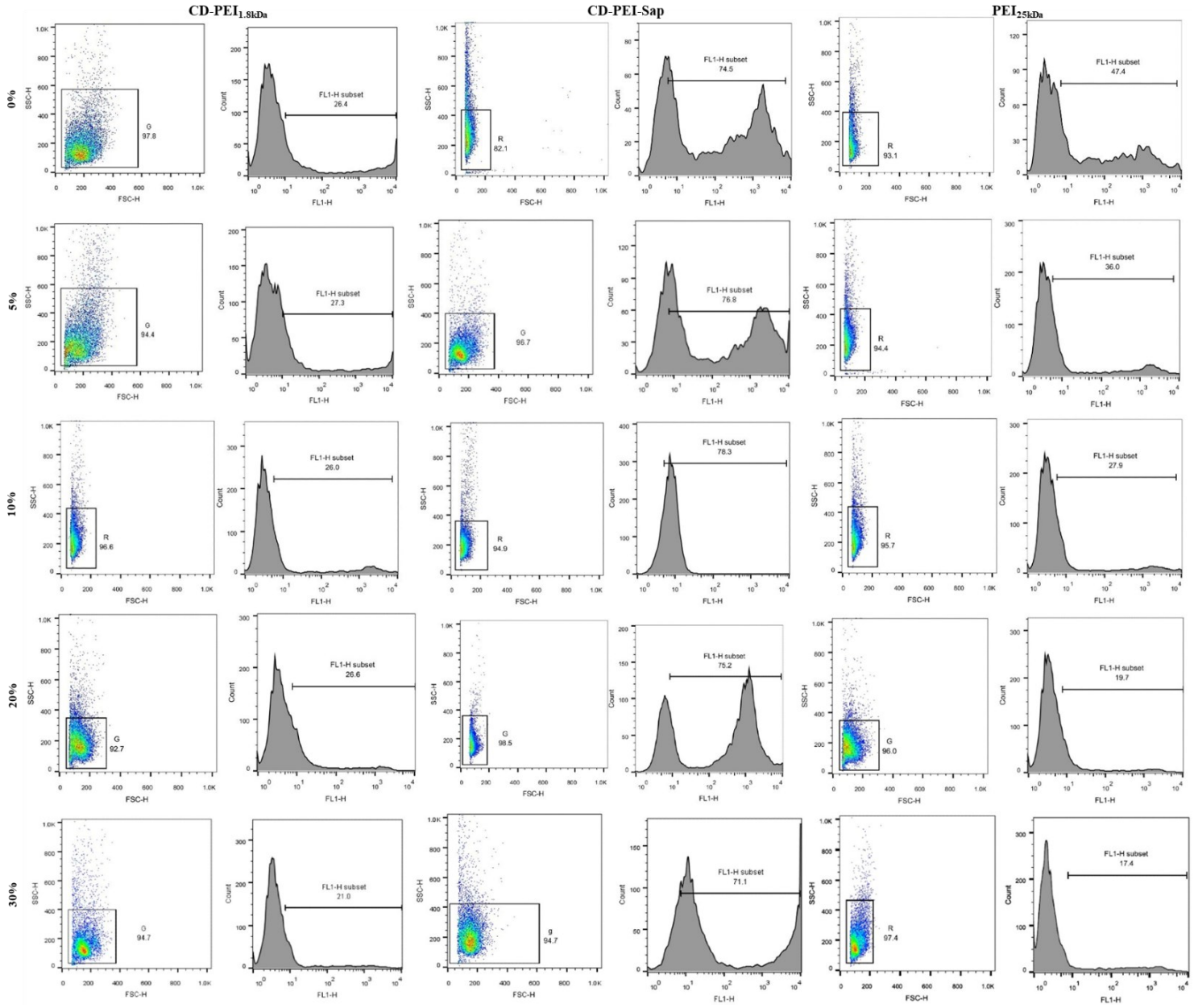
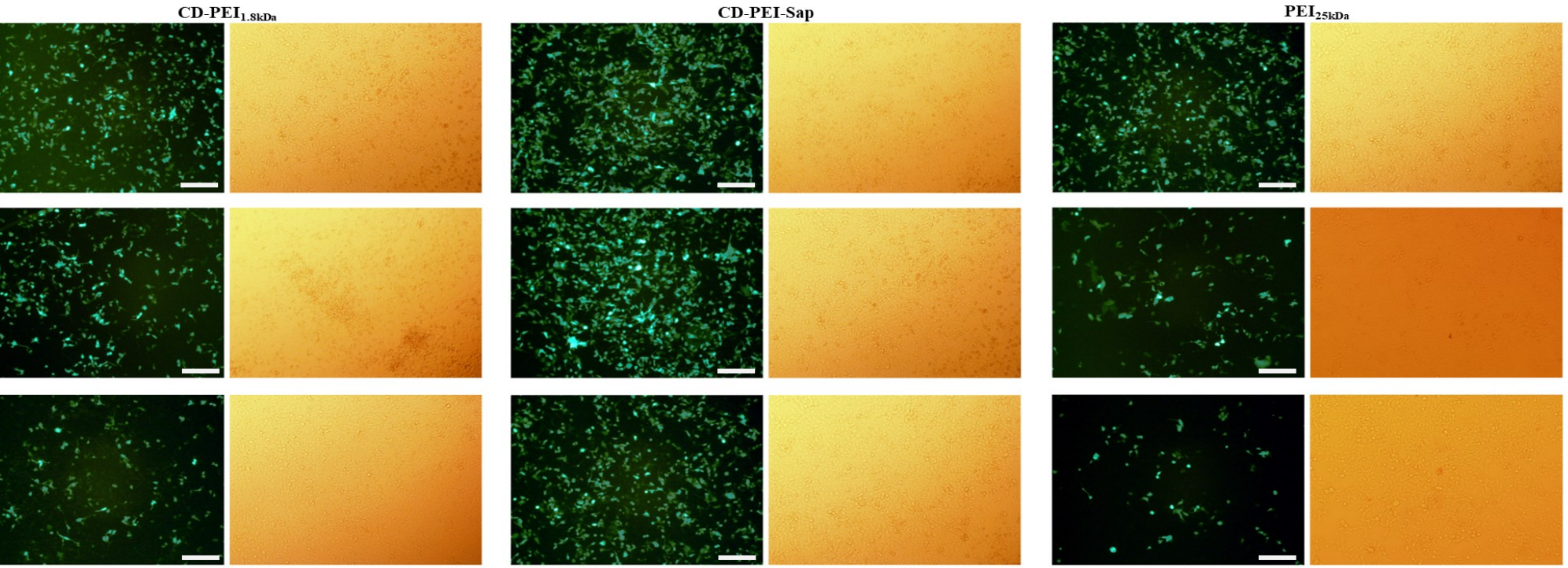
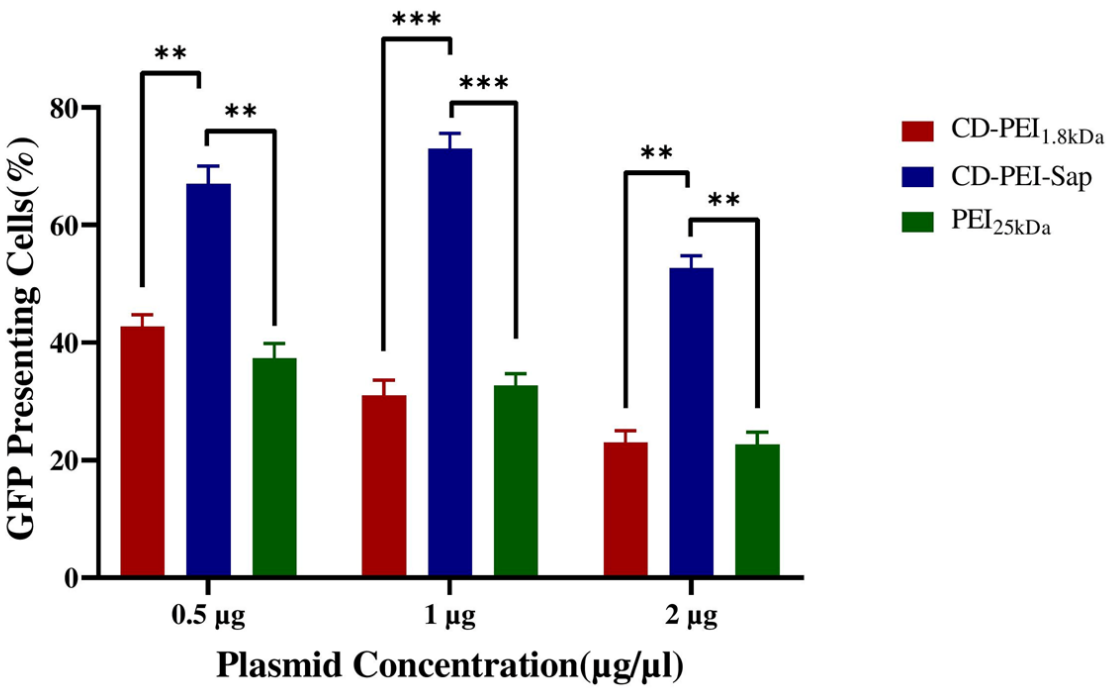


Fig S3: CD-PEI-Sap transfection yield at increasing concentrations of serum. (A) Representative fluorescence microscopy images showing the expression of GFP in HEK 293T cells transfected with CD-PEI $_{1.8kDa}$, CD-PEI-Sap, PEI$_{25kDa}$ at their optimal weight ratios in various concentrations of FBS (0%, 10%, 20% and 30%). (B) Quantitative analysis of transfection efficiency provided as the percentage of GFP-positive cells following transfection with vector/p-CRISPR (3 kb) complexes under identical serum conditions. Transfections were performed using 1 µg of plasmid DNA and evaluated 48 h post-transfection. (Scale bar = 100 µm) CD–PEI–Sap maintained high transfection efficiency even at elevated serum concentrations, indicating boosted serum stability compared to PEI $_{25kDa}$, whose performance decreased with rising serum levels. C) Average flow cytometry data for different serum concentrations.


CD-PEI1.8kDa
CD-PEI-Sap
PEI25kDa

B)


**
**
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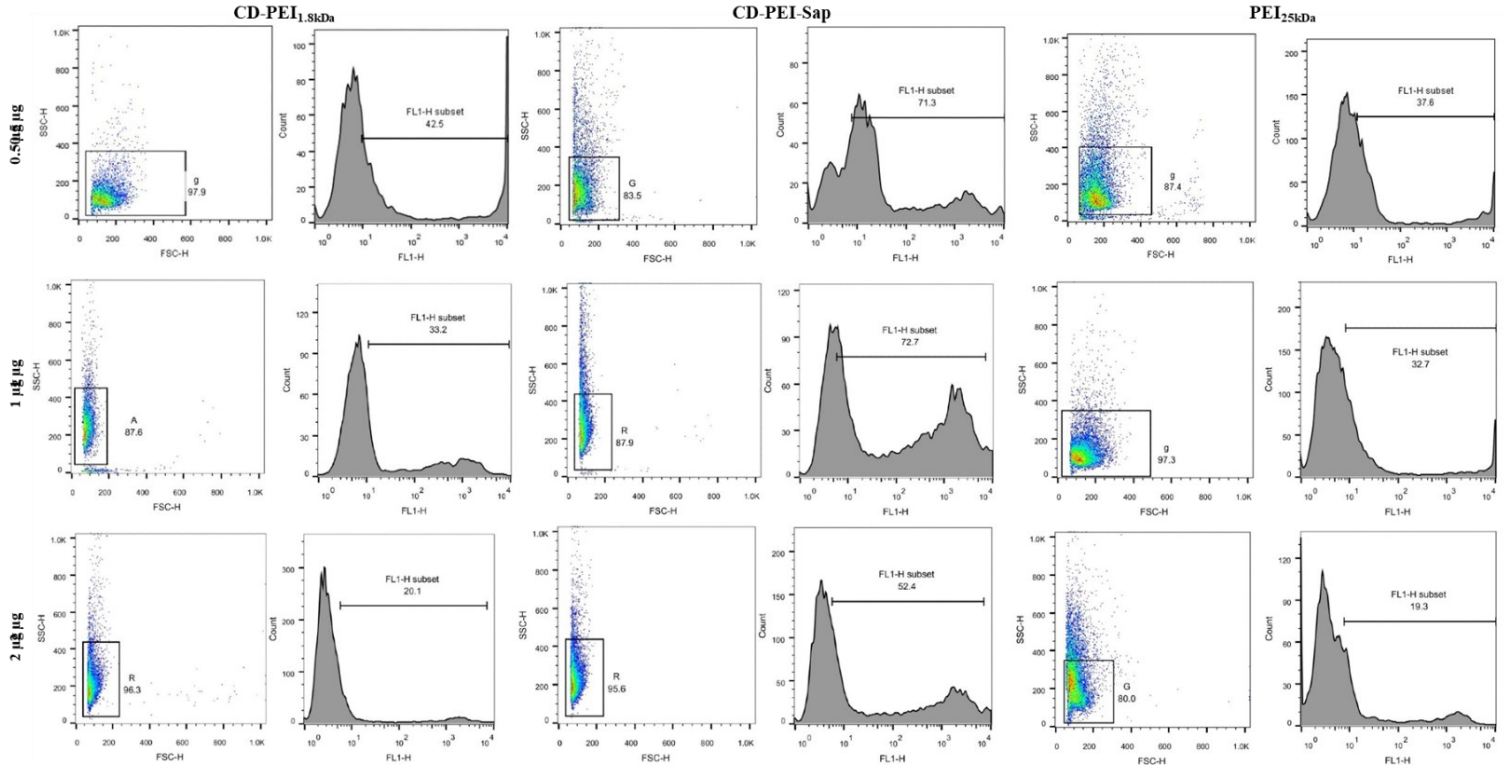
***
**
**
80
60
40
20
0
GFP Presenting Cells(%)
0.5 µg
1 µg
2 µg
Plasmid Concentration(µg/µl)
CD-PEI1.8kDa
CD-PEI-Sap
PEI25kDa

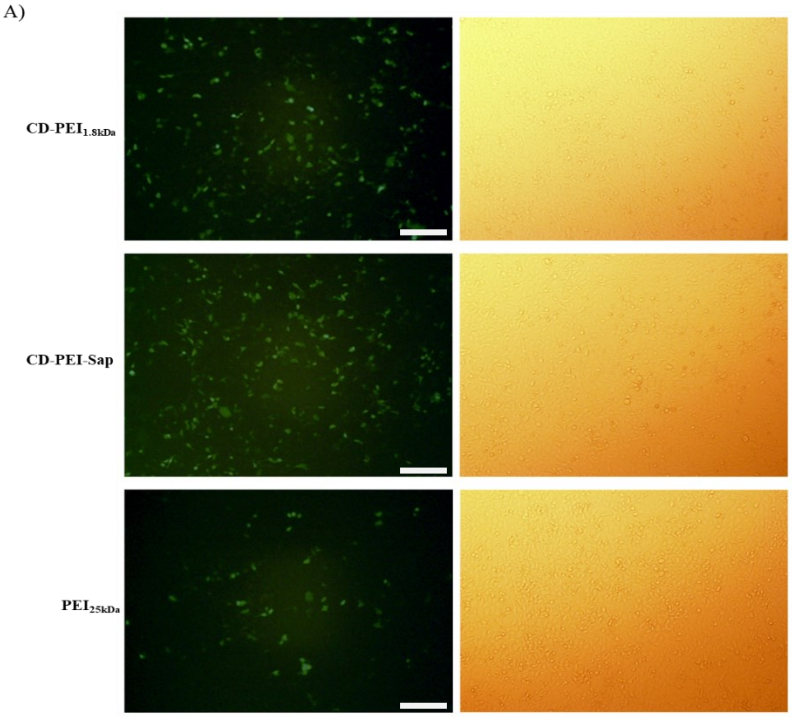
Fig S4: The transfection efficiency of CD-PEI-Sap was evaluated at low doses of p-CRISPR (3kb). (A) The transfection efficiency of CD-PEI $_{1.8kDa}$, CD-PEI-Sap and PEI_{25kDa} at the optimized weight ratio was

C)

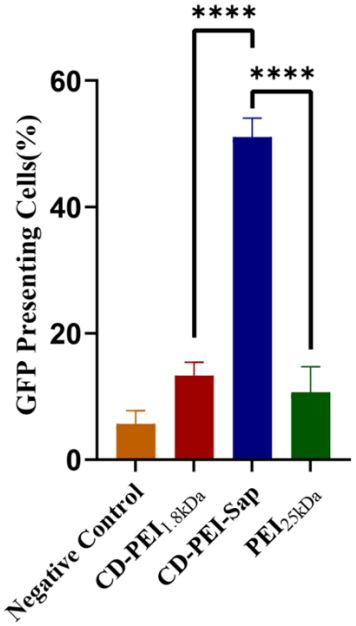


evaluated using fluorescence microscopy images. (B) Quantitative analysis of transfection efficiency expressed as the percentage of GFP-positive cells following transfection with carrier/p-CRISPR (3 kb) complexes at diverse plasmid doses (0.5, 1, and 2 µg). Transfections were evaluated 48 h post-incubation. (Scale bar = 100 µm) CD–PEI–Sap revealed high transfection efficiency even at the lowest plasmid dose (0.5 µg), outperforming $PEI_{25\,kDa}$ and demonstrating efficient gene delivery at reduced DNA concentrations. C) Average flow cytometry information for low doses p-CRISPR.

A)


CD-PEI1.8kDa
CD-PEI-Sap
PEI25kDa

B)


****
****
60
40
20
0
GFP Presenting Cells(%)
Negative Control
CD-PEI1.8kDa
CD-PEI-Sap
PEI25kDa

C)

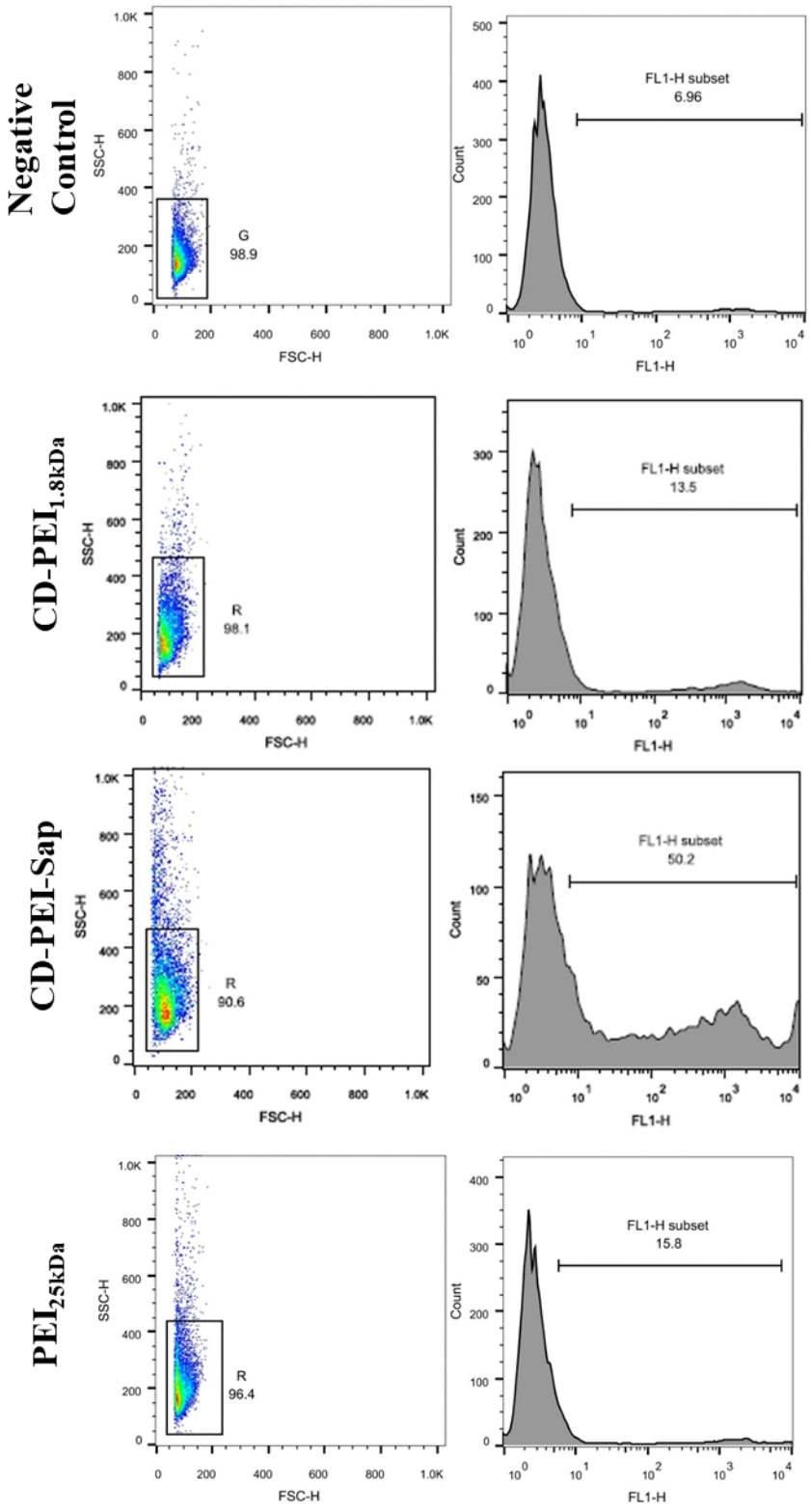

Negative Control
SSC-H
FSC-H
G
98.9
Count
FL1-H
FL1-H subset
6.96
CD-PEI1.8kDa
R
98.1
FL1-H subset
13.5
CD-PEI-Sap
R
90.6
FL1-H subset
50.2
PEI25kDa
R
96.4
FL1-H subset
15.8

Fig S5: Delivery of large p-CRISPR plasmid (9 kb) by CD–PEI–Sap nanocarriers in HEK 293T cells. A) Representative fluorescence microscopy images showing GFP expression after transfection of p-CRISPR (9 kb) into HEK 293T cells 48 h after transfection with a large p-CRISPR plasmid (9 kb) delivered by CD–PEI–Sap nanoparticles. (Scale bar = 100 μm) B) Quantitative assessment of transfection efficiency determined by flow cytometry following delivery of the p-CRISPR (9 kb) plasmid using CD–PEI–Sap at its adjusted carrier-to-plasmid weight ratio (80:1). Scale bar = 100 μm. Despite the large plasmid size, CD–PEI–Sap achieved notable gene delivery, demonstrating its capability to transport high-molecular-weight genetic cargos. C) Average flow cytometry data of p-CRISPR plasmid (9kb) in HEK 293T cells.