Electronic Supplementary Information for

Peptide Amphiphiles with Multifunctional Fragments Promoting Cellular Uptake and Endosomal Escape as Efficient Gene Vectors

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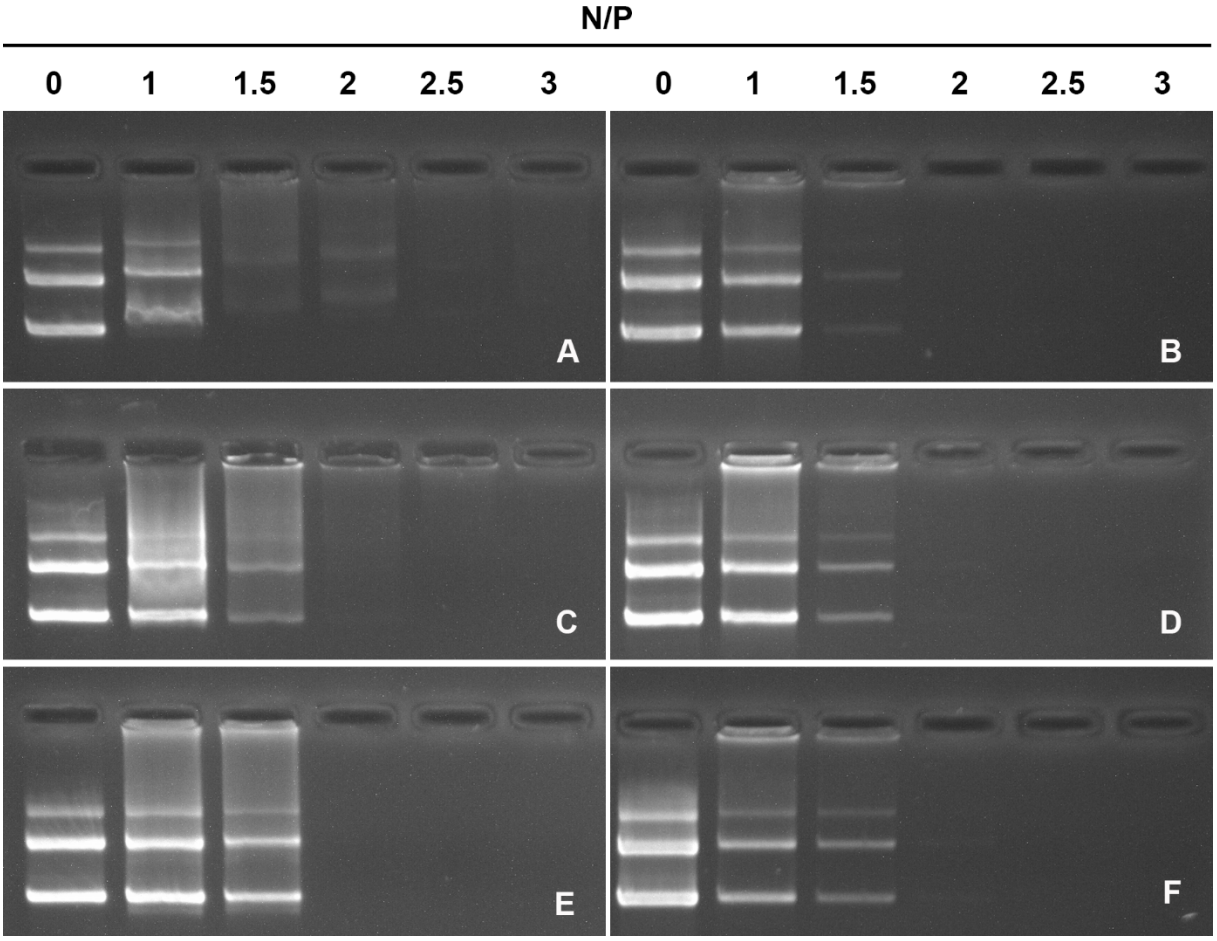

Fig. S1. Agarose gel electrophoresis retardation assay with different N/P ratios for (A) TAT, (B) C_{18}-TAT, (C) $G(LLKK)_3G$, (D) C_{18}- $G(LLKK)_3G$, (E) $C(LLKK)_3C$, and (F) C_{18}-$C(LLKK)_3C$ peptides.

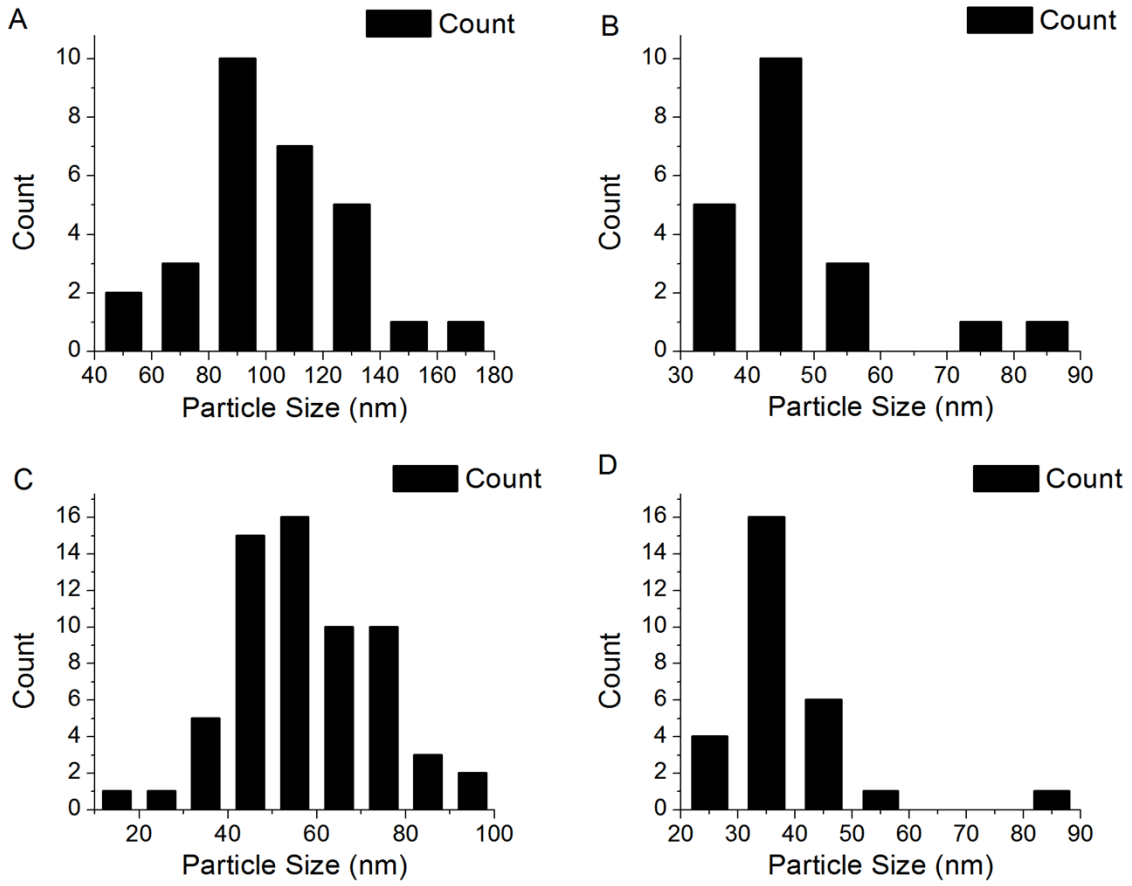

Fig. S2. Size distribution analysis of peptide/DNA nanoparticles. (A) G(LLKK)$_3$G-TAT/DNA, (B) C(LLKK)$_3$C-TAT/DNA, (C) C_{18}-G(LLKK)$_3$G-TAT/DNA, and (D) C_{18}-C(LLKK)$_3$C-TAT/DNA.

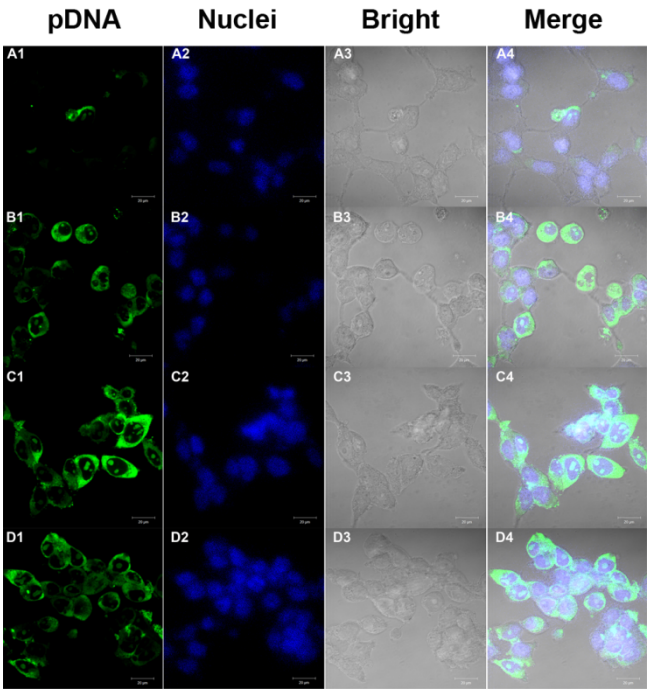

Fig. S3. Confocal microscopy images of the cellular uptake of the following peptide/plasmid DNA (pDNA) complexes labeled with YOYO-1 at a N/P ratio of 4 in 293T cells: (A) $G(LLKK)_3G$-TAT/DNA, (B) $C(LLKK)_3C$-TAT/DNA, (C) C_{18}-$G(LLKK)_3G$-TAT/DNA, and (D) C_{18}-$C(LLKK)_3C$-TAT/DNA. Nuclei are stained with DAPI. Scale bar: 20 μm.

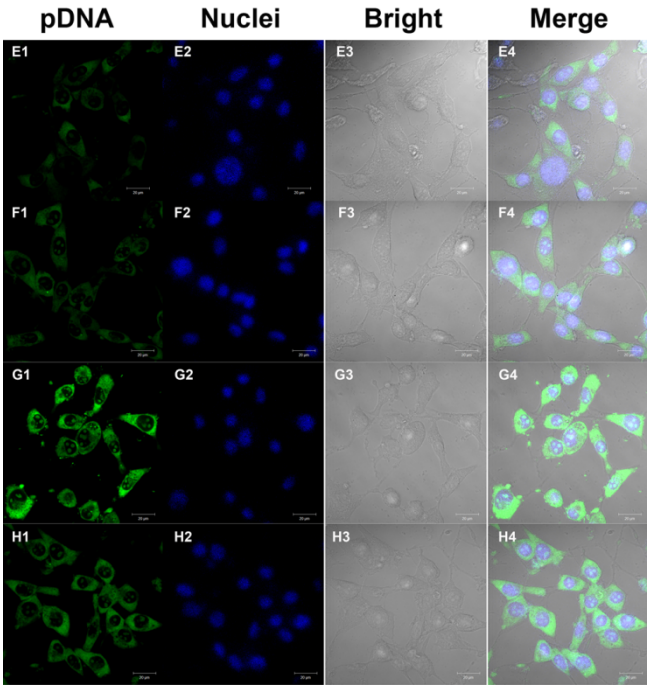

Fig. S4. Confocal microscopy images of the cellular uptake of the following peptide/plasmid DNA (pDNA) complexes labeled with YOYO-1 at a N/P ratio of 4 in NIH-3T3 cells: (A) $G(LLKK)_3G$-TAT/DNA, (B) $C(LLKK)_3C$-TAT/DNA, (C) C_{18}-$G(LLKK)_3G$-TAT/DNA, and (D) C_{18}-$C(LLKK)_3C$-TAT/DNA. Nuclei are stained with DAPI. Scale bar: 20 μm.

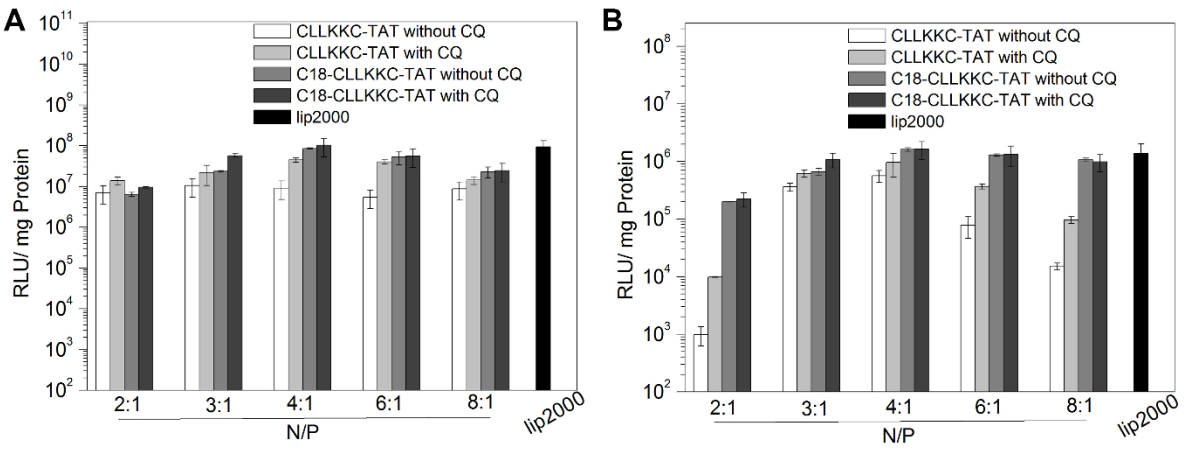

Fig. S5. *In vitro* luciferase expression levels detected in 293T cells (A) and NIH-3T3 cells (B) treated with $C(LLKK)_3C$-TAT and C_{18}-$C(LLKK)_3C$-TAT at N/P ratios ranging from 2 to 8 in the presence or absence of chloroquine (CQ). Data are shown as the mean ± SD (n = 3).

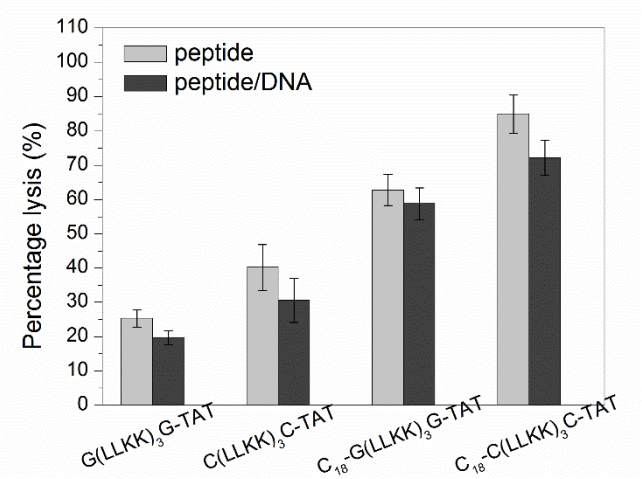

Fig. S6. Liposome leakage assays were performed with peptide and peptide/DNA complexes (at a N/P ratio of 4 and a final concentration of 0.5 μM) and calcein liposomes (POPC/cholesterol = 3:1) in 200 mM NaCl/citrate buffer (PH 5). Fluorescence due to calcein release from liposomes was measured at 515 nm and was plotted as a percentage of total fluorescence detected following treatment with 1% TritonX-100 (positive control). Data shown are the mean ± SD (n = 3).

Table S1. The α-helix content of the peptides studied in a 50% trifluoroethanol (TFE)/PBS solution and a PBS solution.

Peptide	α-helix content	
(each at 50 μM)	50% TFE (%)	PBS (%)
$G(LLKK)_3G$	90.66	16.99
$C(LLKK)_3C$	93.16	27.98
$G(LLKK)_3G$-TAT	70.33	9.24
$C(LLKK)_3C$-TAT	86.48	19.47
C_{18}-$G(LLKK)_3G$	65.56	66.00
C_{18}-$C(LLKK)_3C$	84.28	89.82
C_{18}-$G(LLKK)_3G$-TAT	70.93	42.60
C_{18}-$C(LLKK)_3C$-TAT	76.23	69.42