Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

A self-assembling peptidic platform for the delivery of oligonucleotides to the cell nucleus

Shabnam Tarvirdipour[a,b,c], Michal Skowicki[a], Cora-Ann Schoenenberger[a], Larisa E. Kapinos[d], Roderick Y. H. Lim[d], Yaakov Benenson[c]*, Cornelia G. Palivan[a,b]*

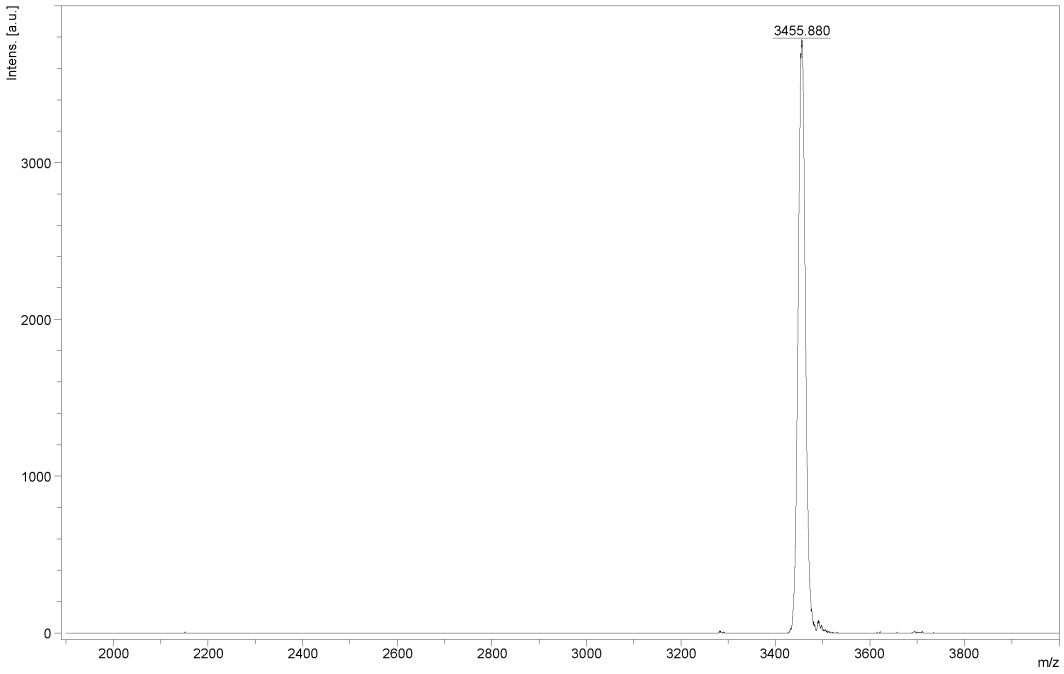

Figure S1. MALDI-TOF mass spectrum of NLS-peptide.

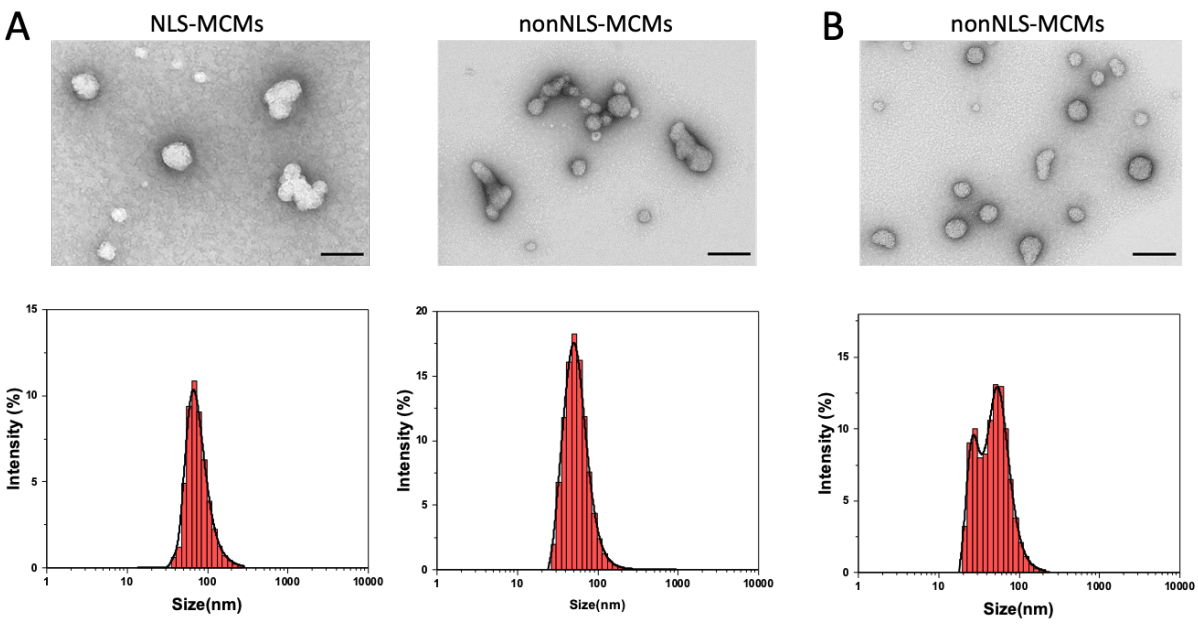

Figure S2. Ultrastructural morphology and size distribution of self-assembled NLS- and nonNLS-MCMs with different DNA payloads, A) scrambled, and B) G3139-GAP ASO. Scale bar, 100nm.

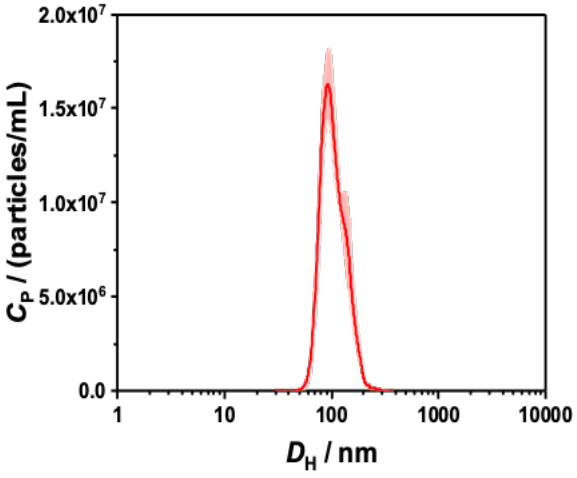

Figure S3. Hydrodynamic diameter of 22nt ssDNA-loaded NLS-MCMs determined by NTA**.**

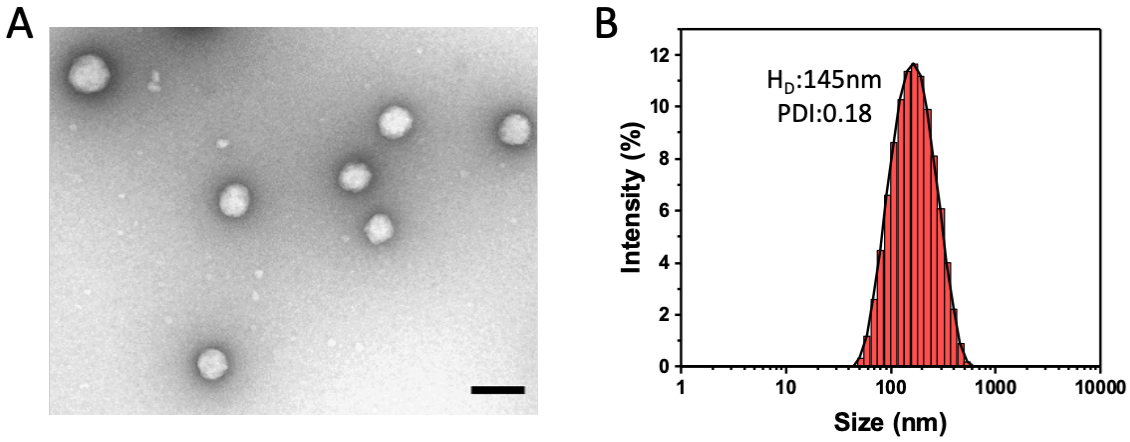

Figure S4. Characterization of self-assembled DNA-free NLS-MCMs. A) TEM, and B) DLS. Scale bar, 200nm.

Table S1. Characterization of NLS- and nonNLS-MCMs loaded with different oligonucleotides, suspended in water, at pH 7.

MCM-NPs	PDI	D_H(nm)	Zeta potential(mV)
Scrambled oligo loaded NLS-MCMs	0.19	76±2	21±7
G3139-GAP loaded nonNLS-MCMs	0.28	52±14	10±4
Scrambled oligo loaded nonNLS-MCMs	0.18	58±8	8±3

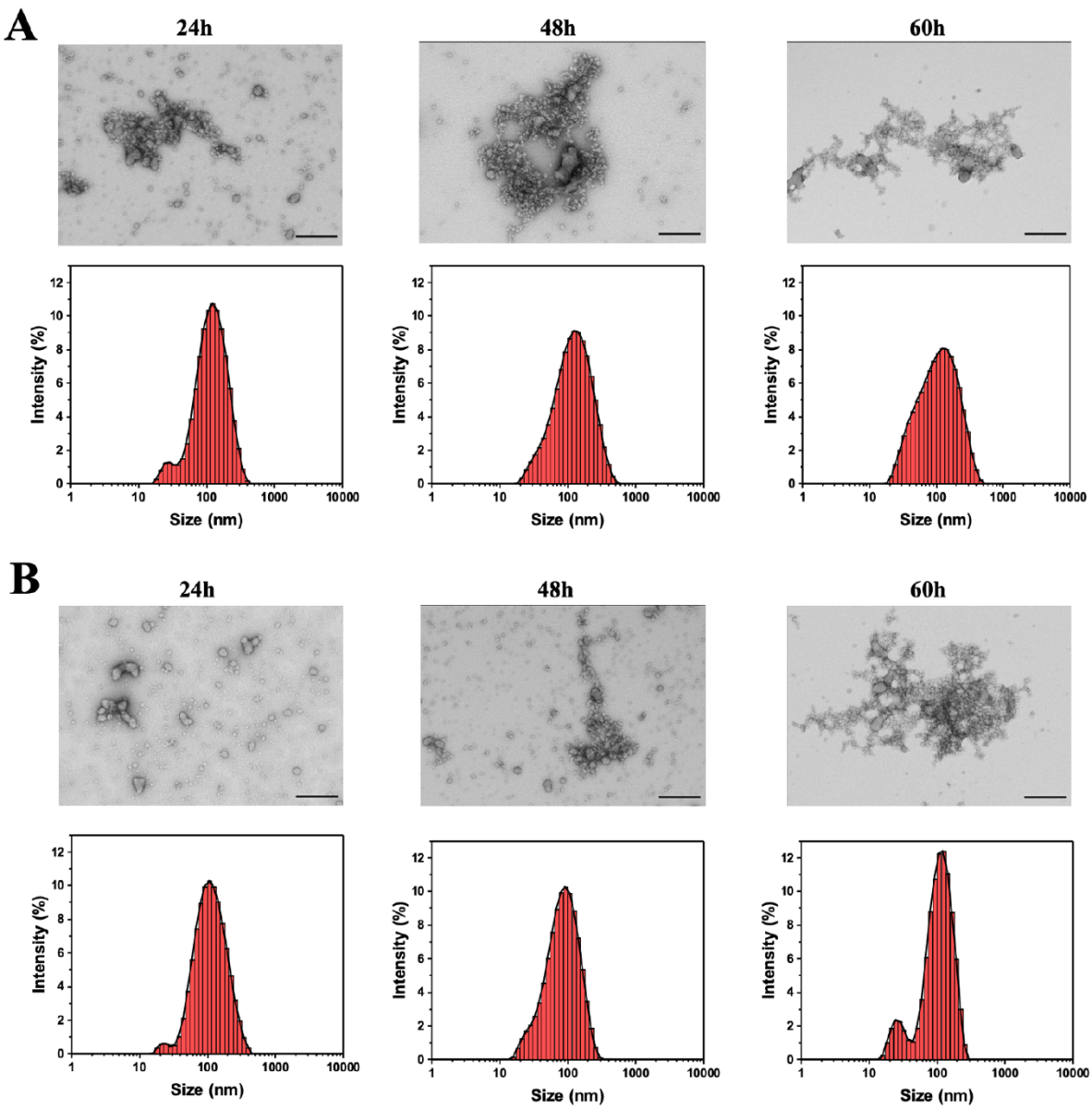

Figure S5. Thermo-responsiveness of NLS-MCMs. TEM and DLS analysis of NLS-MCMs A) with DNA, and B) without DNA after 24, 48 and 70 h incubation at 37 °C. Scale bars = 200 nm.

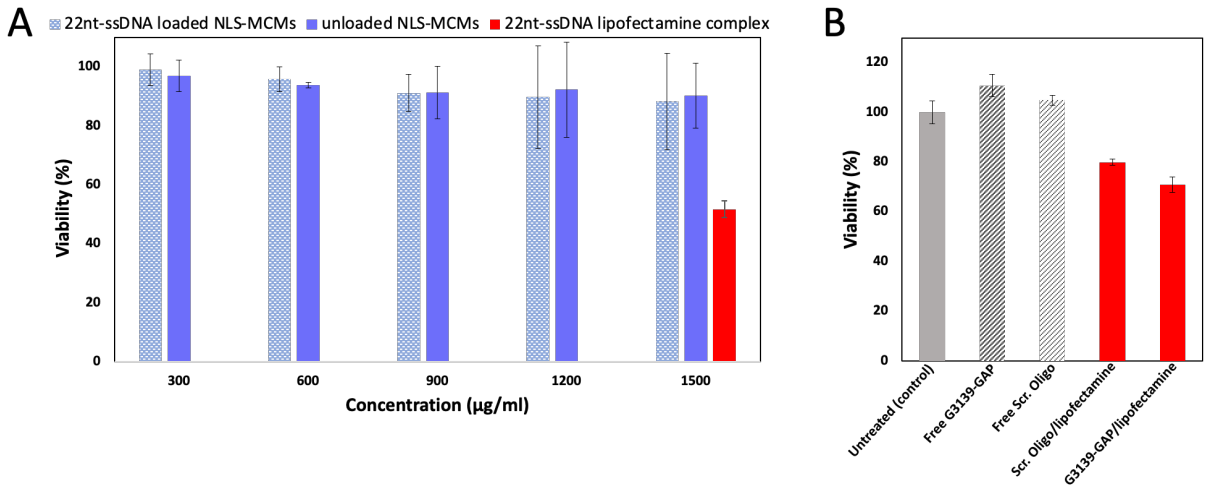

Figure S6. A) Effect of DNA-free and 22nt ssDNA-loaded NLS-MCMs on MCF-7 cell proliferation at different peptide concentrations after 24 h of exposure. Comparison at 1500µg/ml peptide concentration to lipofectamine complex with corresponding 22nt ssDNA concentration. B) lipofectamine-mediated transfection of scrambled oligonucleotides and G339-GAP ASO after 48 h of exposure.

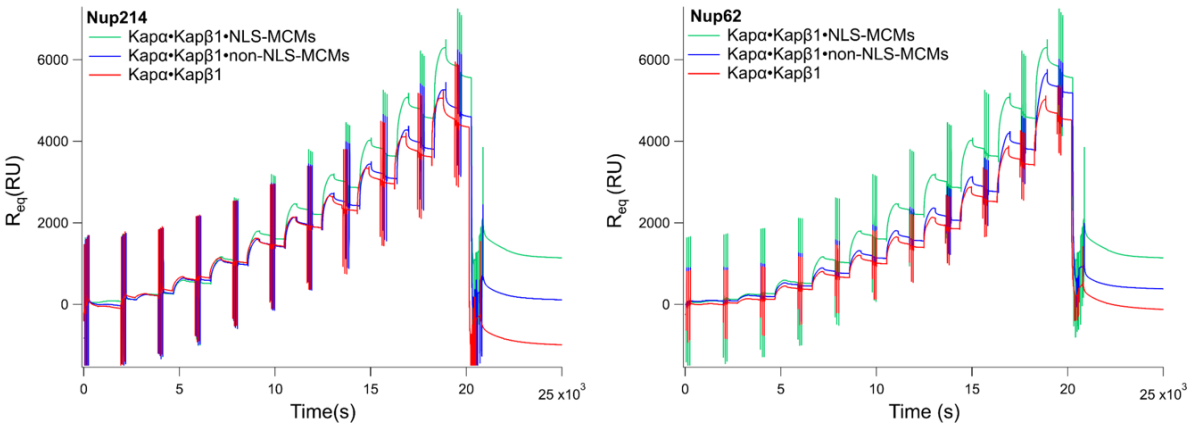

Figure S7. SPR sensograms resolve the binding interactions of Kapα•Kapβ•NLS-MCMs, Kapα•Kapβ1•nonNLS-MCMs, and standalone Kapα•Kapβ1 to Nup214 (left) and Nup62 (right).

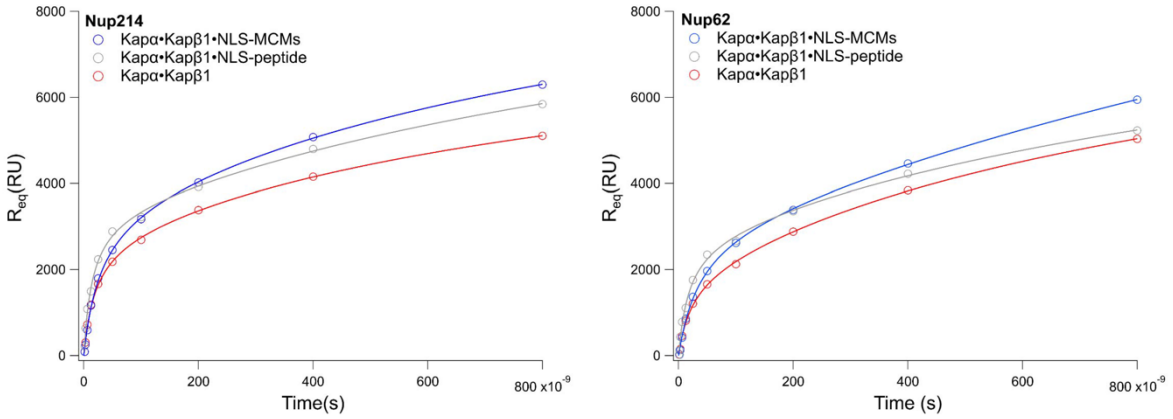

Figure S8. Langmuir isotherm fits (solid lines) for Kapα•Kapβ1•NLS-MCMs compared to free NLS-peptide. MCM nanocarriers elicit the highest FG Nup-binding response compared to free peptide as measured by SPR.

Table S2 Maximal SPR response signals and equilibrium dissociation constants of free peptide/karyopherin complexes binding to Nups.

		R_{max1} (RU)	R_{max2} (RU)	K_{D1} (nM)	K_{D2} (nM)
Kapα•Kapβ1 / NLS (free peptide)	**Nup214**	3282.8±286	7809.3±3870	14.6±2.5	1574.6±1340
	Nup62	2706±283	7122±2700	17.7±3.4	1396.3±990
Kapα•Kapβ1 / nonNLS (free peptide)	**Nup214**	2814.9±384	5748.8±1690	14.1±3.6	878.8±637
	Nup62	1804.6±226	4764.2±485	14.5±3.2	637.2±216
Kapα•Kapβ1	**Nup214**	2672±387	5711.4±1850	19.4±4.8	1028.1±779
	Nup62	1895±229	7495.2±1420	22.4±5.7	1077.3±477

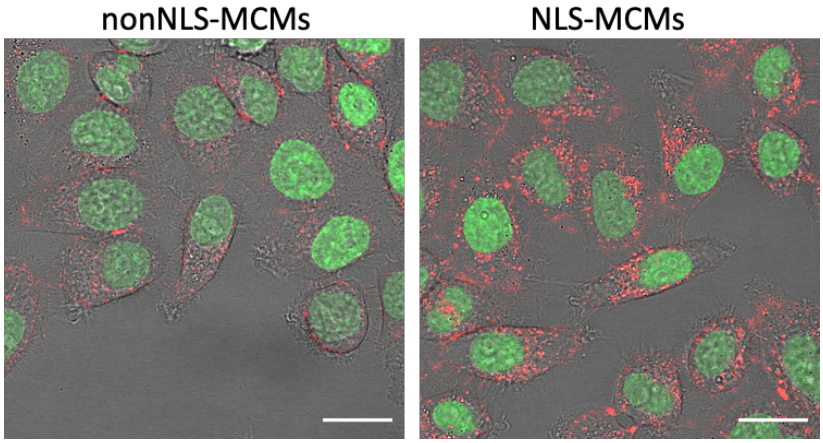

Figure S9 CLSM merged images of H2B-GFP expressing HeLa cells recorded after 5 h incubation with DNA-loaded NLS- and nonNLS-MCMs. Scale bars, 20µm.

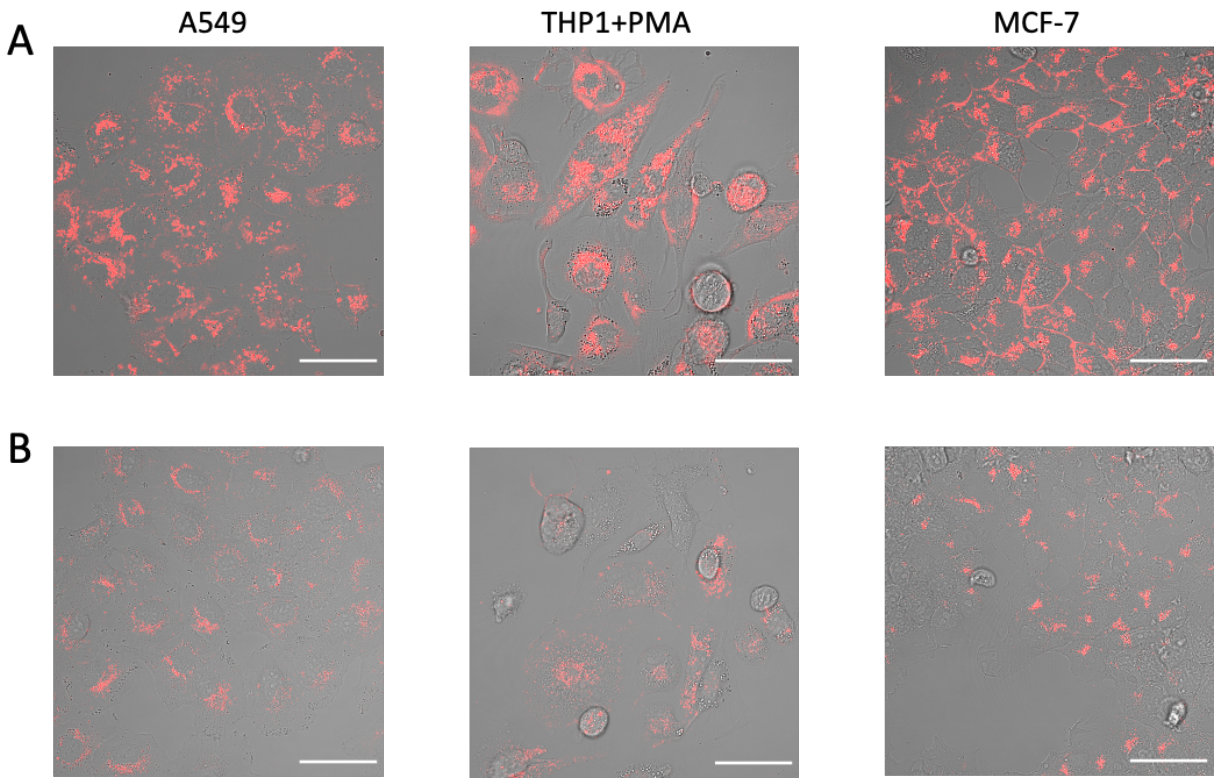

Figure S10. CLSM merged images of A549, THP-1stimulated with PMA and MCF-7 cells recorded after 24 h incubation with G3139-GAP-loaded A) NLS-MCMs, and B) nonNLS-MCMs. Scale bars = 50µm.

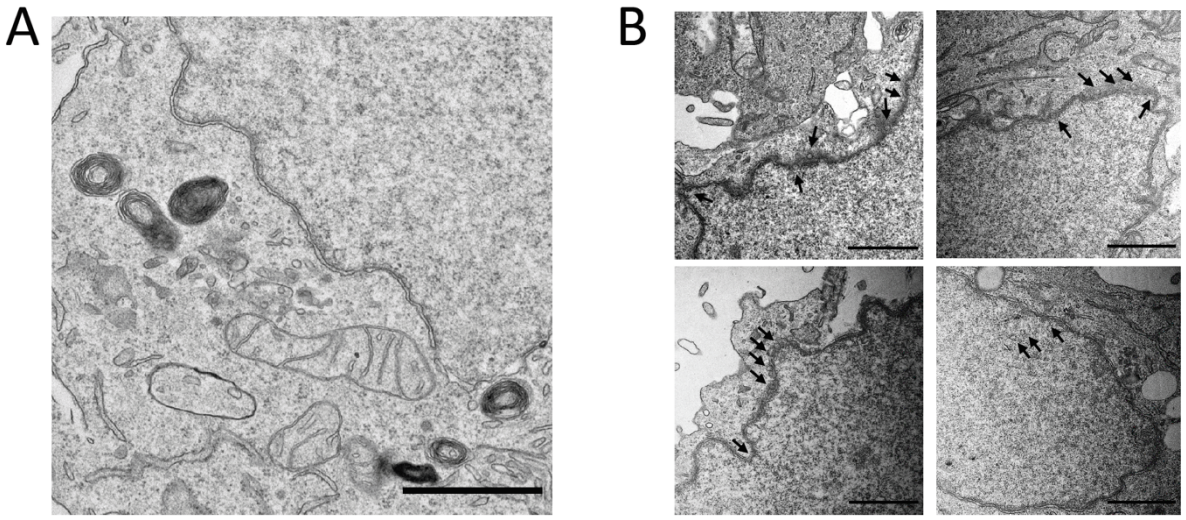

Figure S11. Ultrastructural analysis of A) untreated cells (control), B) cells treated with DNA-loaded NLS-MCMs, Scale bars, 1µm.

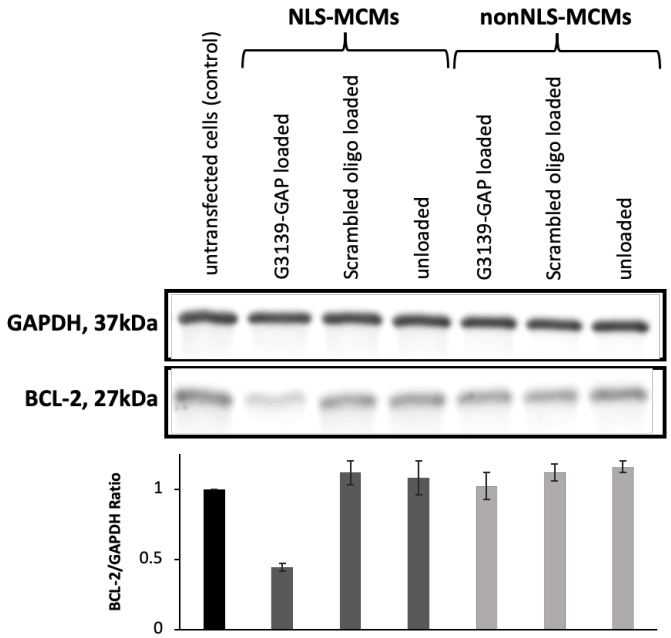

Figure S12. Western blot analysis of BCL-2 proteins in 6μg of MCF-7 total cell extract after 24 h. GAPDH was used as loading control. Quantitative densitometry of the immunoblots are mean ± s.d. of the relative intensity of the bands from three independent assays; nonNLS-MCM-treated (dark grey bars), NLS-MCM-treated (dark grey bars), untreated (black bar).

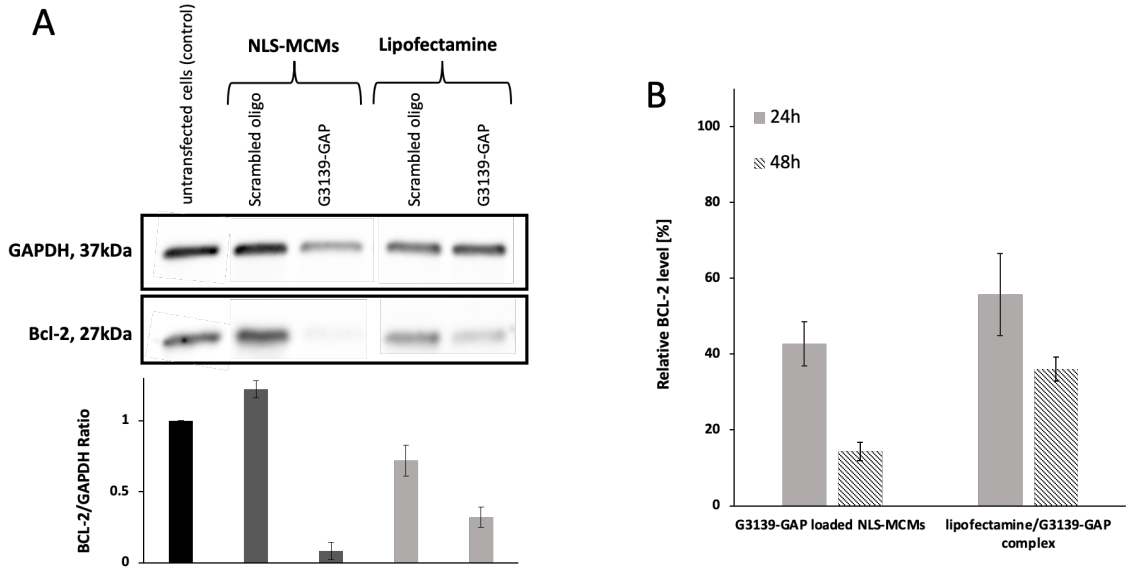

Figure S13. Therapeutic effects of cells transfected with lipofectamine/G3139-GAP complex. A) Western blot analysis of BCL-2 proteins in 6μg of MCF-7 total cell extract after 48 h. GAPDH was used as loading control. B)

Quantitative densitometry of the immunoblots. Lipofectamine-treated (light grey bars), NLS-MCM-treated (dark grey bars), untreated (black bar). C) BCL-2 expression levels in cells transfected with G3139-GAP relative to scrambled oligo delivered with NLS-MCMs or lipofectamine complexes. Data are mean±s.d. of the relative intensity of the bands from three independent assays.