Electronic Supplementary Material (ESI) for Nanoscale.

CDC20siRNA and paclitaxel co-loaded nanometric liposomes of nipecotic acid-derived cationic amphiphile inhibit xenografted neuroblastoma

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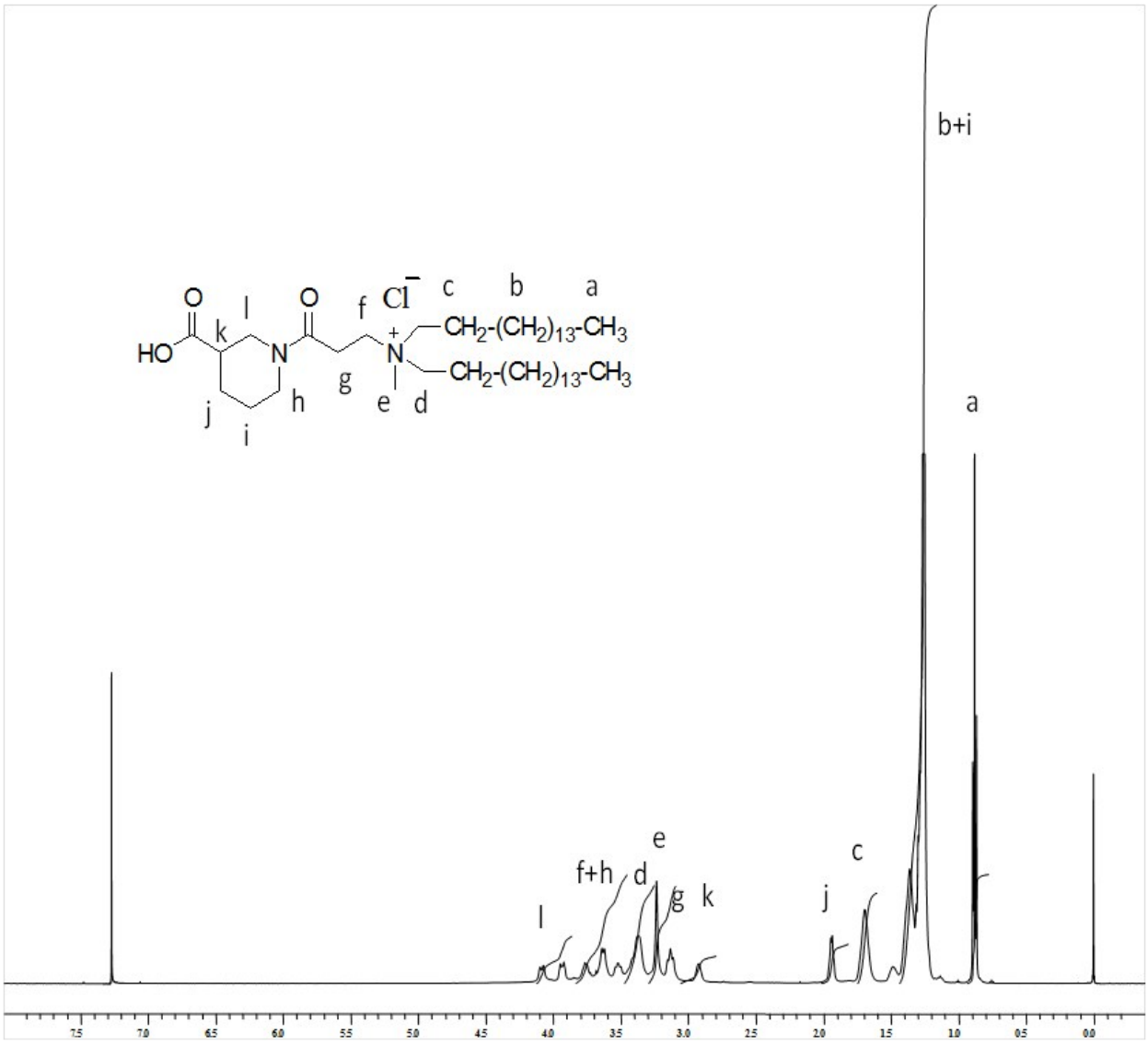

Fig. S1. [1]H-NMR spectrum of **NACA**

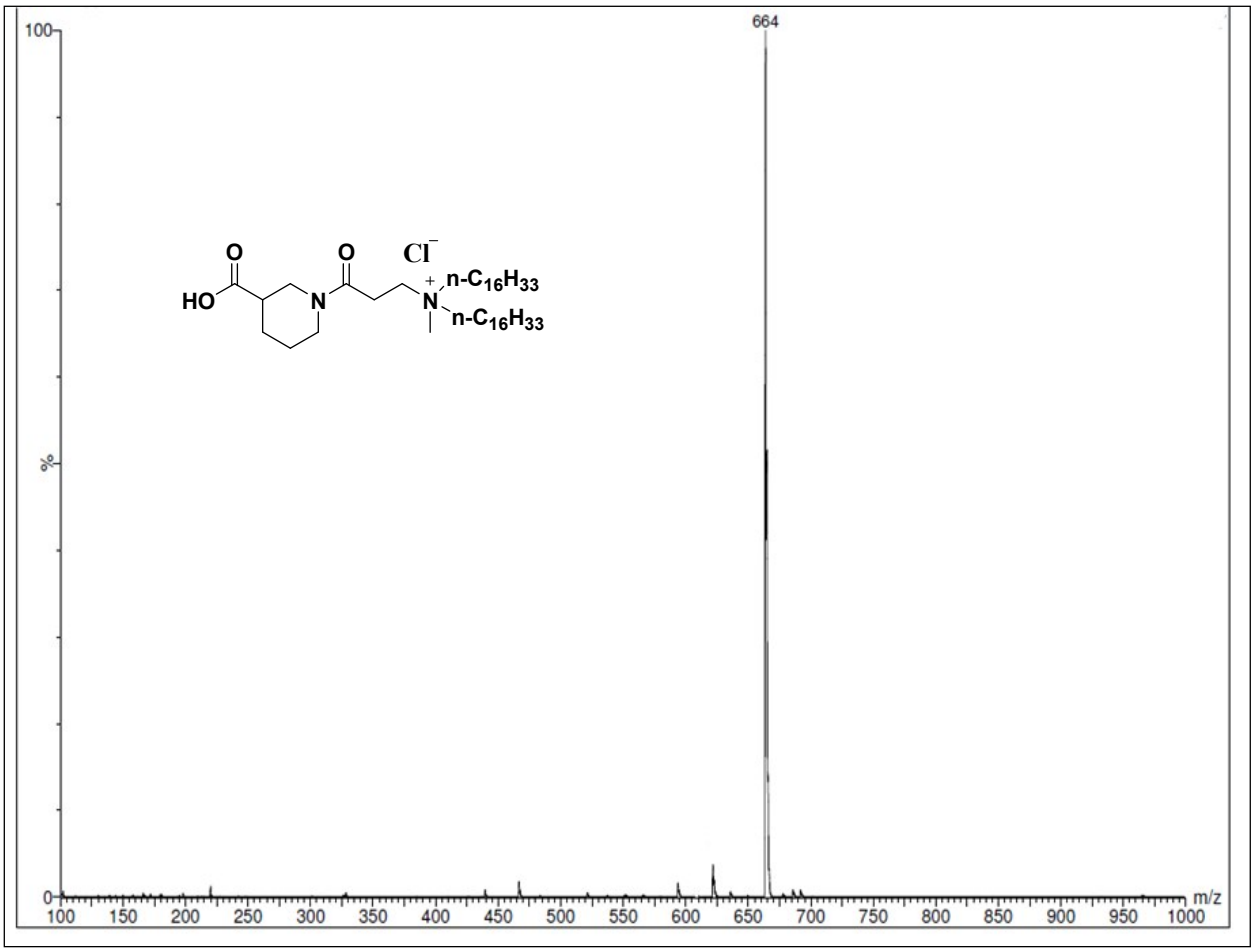

Fig. S2. ESI-Mass spectrum of **NACA**

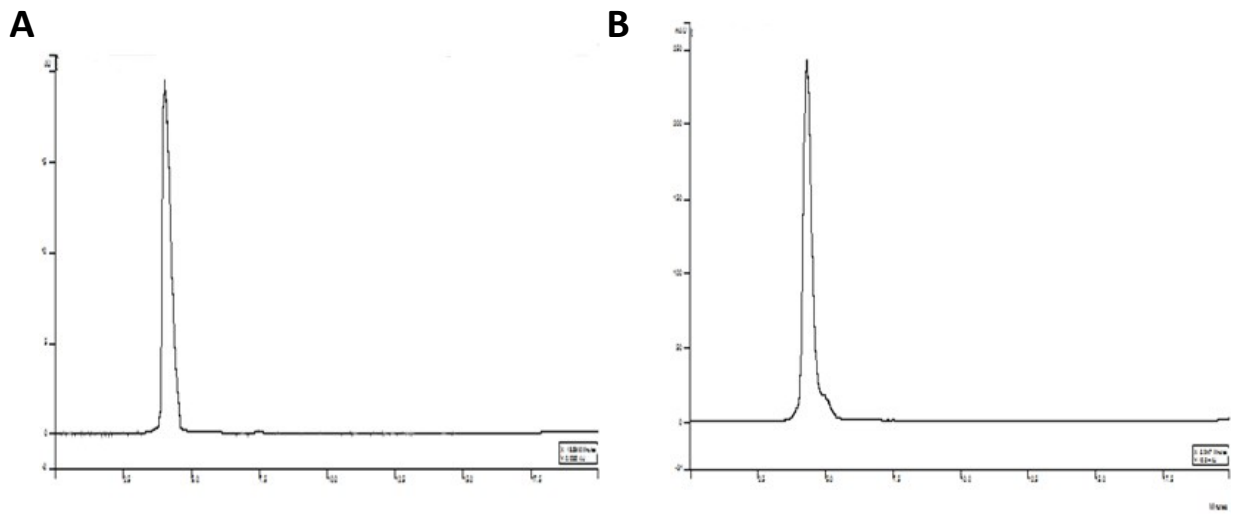

Fig. S3. HPLC Chromatograms of **NACA** using Mobile Phases: pure Methanol (**A**); Methanol:Water, 95:5 (v/v) (**B**).

HPLC Conditions:

System: Varian Prostar 210
Column: Metasil AQ 10U C18 120A, 250 x 10 mm
Flow Rate: 1.0 mL/min (0-20 min)
Typical Column Pressure: 60-65 Bars
Temperature: 25 ^{0}C; Detection: UV at 260 nm

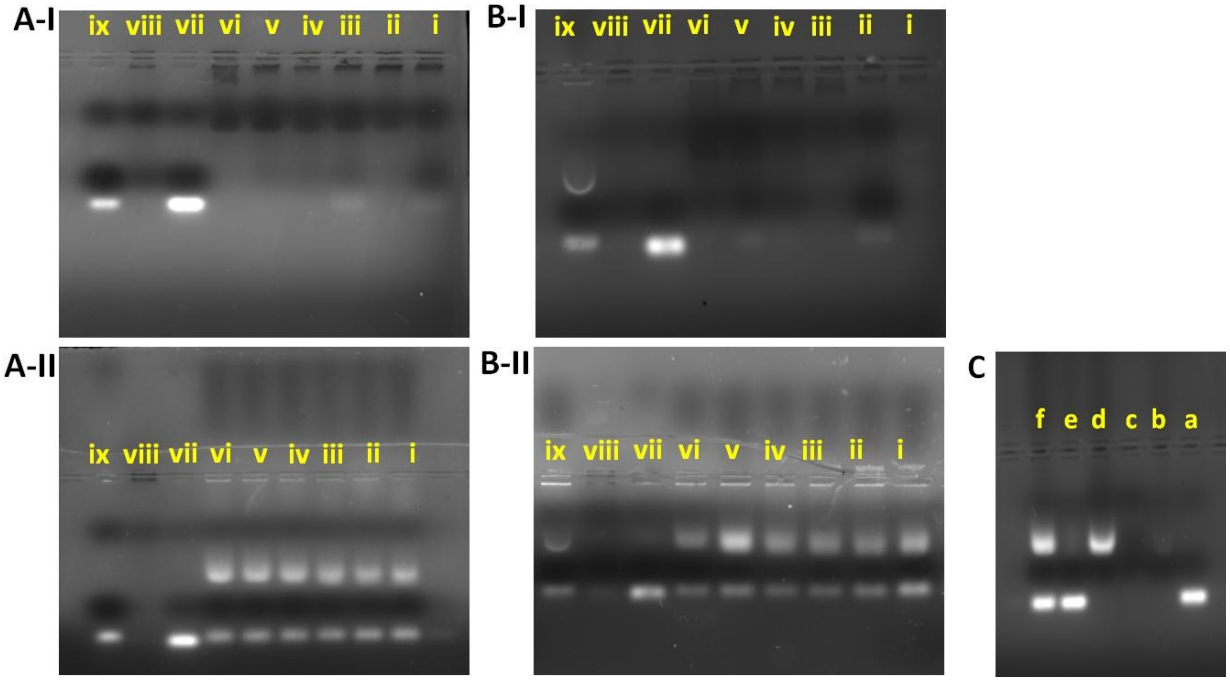

Fig. S4: Serum stability of FAM-siRNA encapsulated liposomes of NACA & NTL**.** Gel electrophoretic mobility profiles of FAM-siRNA encapsulated liposomal samples of NACA (A-I) and NTL (B-I) after 24 h incubation with increasing concentrations (10-60%) of added serum, FAM-siRNA loaded liposomal samples of NACA (A-II) and NTL (B-II) were first incubated with increasing concentrations (10-60%) of added serum for 24 h and then treated with 0.5% SDS for 20 min. For gels in Parts **A-I**, **B-I**, **A-II** & **B-II**: 10% added FBS (lane i), 15% added FBS (lane ii), 20% added FBS (lane iii), 30% added FBS (lane iv), 40% added FBS (lane v), and 60% added FBS (lane vi); naked FAM-siRNA (lane vii); siRNA loaded liposomes without incubation with serum (lane viii); and siRNA loaded liposomes treated with SDS (lane ix). **C**. The slower migrating bands observed in gels A-II & B-II originate from complexes of SDS & serum ingredients. naked FAM-siRNA (lane a), 0.5% SDS (lane b), 40% serum (lane c), 0.5% SDS mixed with 40% serum (lane d), 0.5% SDS mixed with siRNA (lane e), and 0.5% SDS mixed with siRNA and 40% serum (lane f).

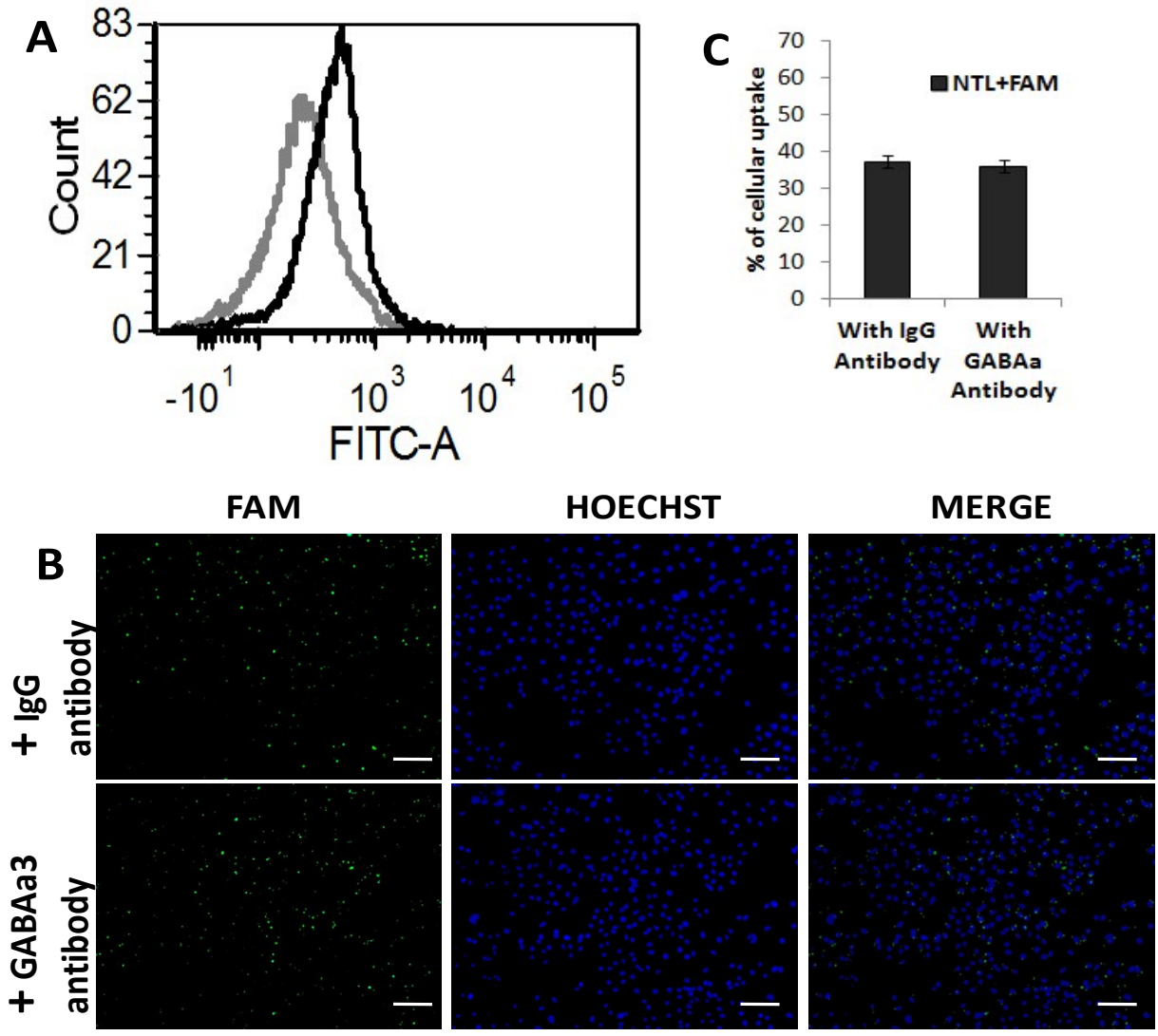

Fig. S5. (**A**) Expression profile of GABAa3 receptor in IMR-32 cells by flow cytometry. The gray trace on the left side represents the signal of the control isotype antibody and the black trace on the right represents the signal from IMR-32 cells treated anti-GABAa3 antibody. (**B**) Epifluorescence micrographs (10X) of IMR-32 cells treated with FAM-siRNA encapsulated non-targeting liposomes (**NTL**) using IMR-32 cells pre-saturated with IgG antibody (upper chanels) & GABAa3 antibody (lower chanels). Bar = 100 μm. (**C**) Quantification of the cellular uptake profiles observed in part **B.** Percentages of cellular uptake [fluorescence intensity ratio for the FAM-

siRNA labeled cells (green) and hoechst-positive cells (blue)] x 100] were measured using Image J Analysis software.

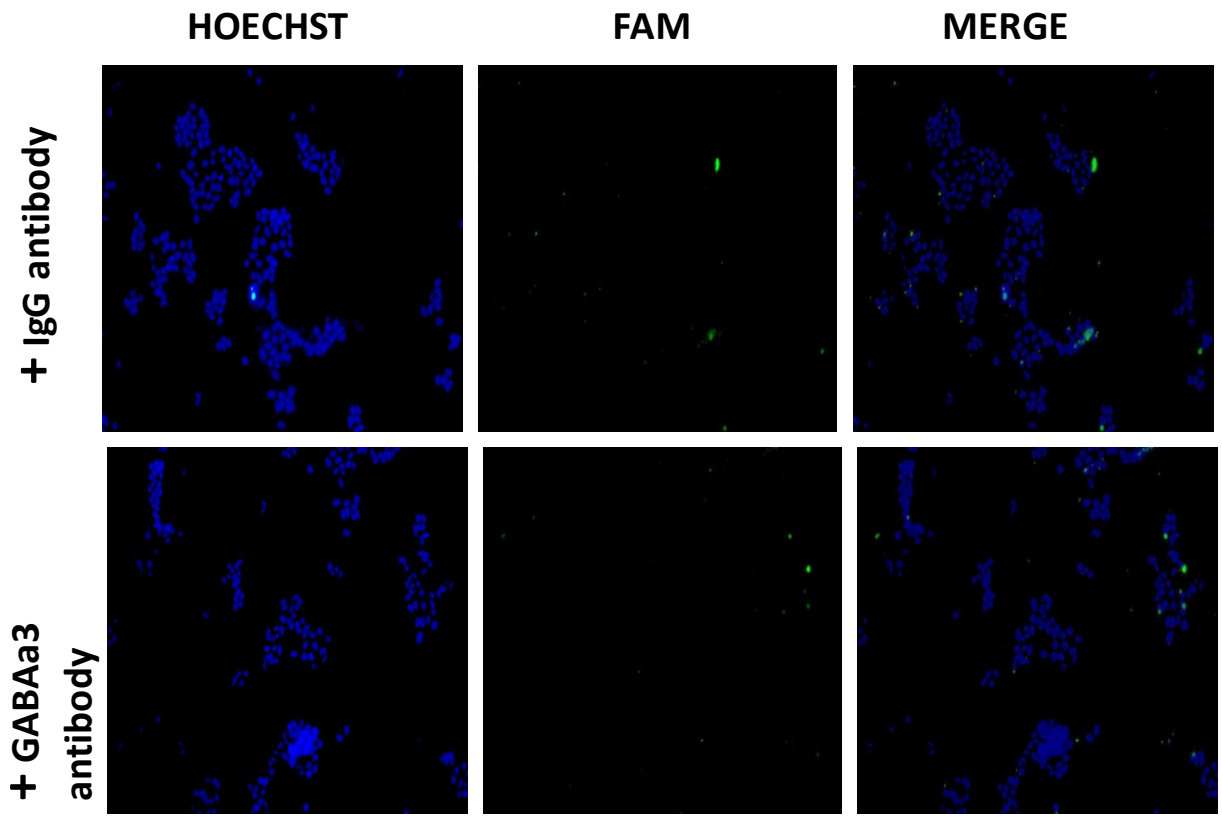

Fig. S6. Epifluorescence micrographs of healthy HEK-293 cells (10X) treated with: FAM-siRNA (green) encapsulated **NTL**-liposomes. HEK-293 cells were pre-saturated with IgG antibody (panel indicated as **+IgG antibody**) and with GABAa3 antibody (panel indicated as **+GABAa3 antibody**).

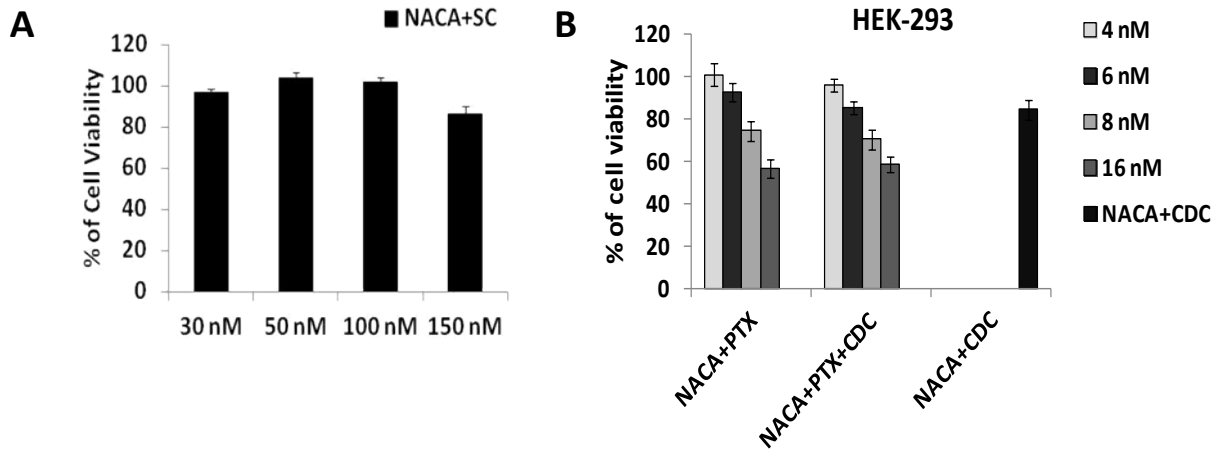

Fig. S7. Percentage cell viability profile of (A) IMR-32 cells treated with NACA liposomes encapsulating scrambled siRNA and (B) HEK-293 cells treated with **NACA** liposomes containing encapsulated: i) indicated varying concentrations of PTX (**NACA+PTX**), ii) 50 nM CDC20siRNA (**NACA+ CDC**), and iii) 50 nM CDC20siRNA plus indicated varying concentrations of PTX (**NACA+PTX+ CDC**).

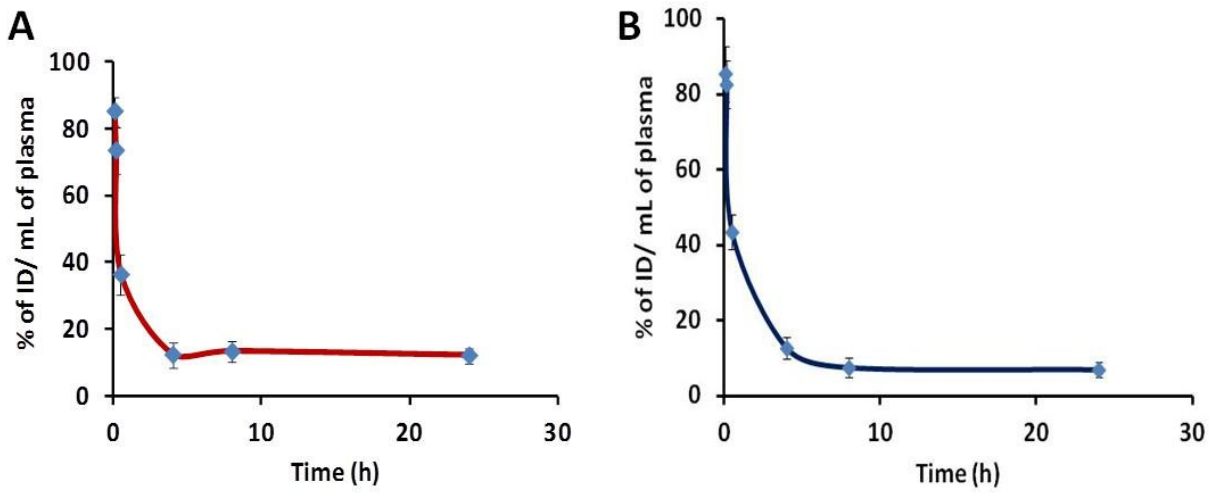

Fig. S8. Plasma stability profiles of i.v. injected FAM-labelled siRNA encapsulated **NACA** liposomes (A) and **NTL** liposomes (B).

Table S1: Zeta potential of liposomal formulations:

Liposomal formulation	Zeta Potential (mV)
NACA	5.5 ± 1.8
NACA + siRNA	4.0 ± 1.2
NACA + PTX	3.4 ± 2.1
NACA + siRNA + PTX	4.8 ± 0.9
NTL	3.9 ± 1.5
NTL + siRNA	4.7 ± 1.3
NTL + PTX	4.1 ± 0.7
NTL + siRNA + PTX	4.9 ± 1.6

Table S2: primer sequences used in RT-PCR experiment

mRNA type	**Primer type**	**Primer sequence**
Human CDC20	Forward	5´TCCAAGGTTCAGACCACTCC 3´
	Reverse	5´GATCCAGGCCACAGAGGATA 3
Human 18S	Forward	5´ GCAATTATTCCCCATGAACG 3´
	Reverse	5´GGCCTCACTAAACCATCCAA 3´