Supporting Information:

General Methods and Materials for Chemical Synthesis. Microwave irradiations were performed in Biotage Initiator. Other reactions were perfomed in capped glass vials. ^{1}H NMR spectra were measured on a 300 & 500 MHz Varian spectrometer using TMS as internal standard. Mass spectra were measured on a Waters Acquity LC-MS instrument. Thin-layer chromatography (TLC) was performed on precoated silica gel GF plates purchased from Merck, Inc. Isco Combiflash systems were used for flash chromatography. All other chemicals were obtained from commercial sources and were used as received.

*Synthesis of compound **6*** (Fig. S1A). Compound **5** (487 mg, 1.02 mmol) was charged in a 10 ml flask and trifluoroacetic acid (TFA, 1.3 mL) was added dropwise at 0 ^{o}C. The reaction mixture was warmed to room temperature and stirred for 30 min. The solvents were evaporated under reduced pressure and the TFA salts in DMF (3.5 mL) were added dropwise to pyridine (100 mL) at 0 ^{o}C. The reaction mixture was slowly warmed to room temperature and stirred for overnight. The solvents were evaporated under reduced pressure and the white solid was washed with EtOAc to give pure **6** in 69% yield. MS: *m/z* 525 ($M+H^+$); ^{1}H NMR (500 MHz, DMSO, ppm): δ 1.29-1.40 (m, 8H, CH_2CH_2), 1.61-1.68 (m, 4H, CH_2), 2.97 (dd, *J* = 6.0, 12.5 Hz, 4H, NCH_2), 3.79 (br, 2H, COC*H*), 7.22 (t, *J* = 5.5 Hz, 2H, aromatic), 7.33-7.37 (m, 8H, aromatic), 8.10 (s, 2H, N*H*).

*Synthesis of compound **7*** (Fig. S1A). A cloudy solution of compound **6** (95 mg, 0.18 mmol) in 50% acetic acid/CH_2Cl_2 (6 mL) was added to Pd on charcoal (10 wt %, 36.5 mg). The black suspension was degassed for 5 mins and hydrogen gas was introduced. The reaction mixture was stirred at rt overnight and was then filtered through a layer of Celite, which was washed several times with MeOH. The combined filtrates were concentrated to obtain yellow viscous oil, which was solidified by adding EtOAc. The solid was washed by ethyl acetate to yield compound **7** in 90% yield. MS: *m/z* 257 ($M+H^+$); ^{1}H NMR (500 MHz, D_2O, ppm): δ 1.39-1.52 (m, 4H, CH_2), 1.67-1.71 (m, 4H, CH_2), 1.84-1.88 (m, 4H, CH_2), 2.99 (t, *J* = 7.5 Hz, 4H, NCH_2), 4.14 (t, *J* = 5.0 Hz, 2H, COC*H*).

*Synthesis of **cKK-E12*** (Fig. S1A). To a mixture of compound **7** (169.2 mg, 0.45 mmol) and 1,2-epoxydodecane (523 mg, 2.7 mmol) in EtOH was added triethylamine (182 mg, 1.8 mmol), which was stirred 30 mins at rt. The reaction mixture was then irradiated in the microwave reactor at 150 ^{o}C for 5 h. The mixture was purified by flash column chromatography to obtain compound ***cKK-E12*** in 52% yield as light yellow oil. MS: *m/z* 993 ($M+H^+$); ^{1}H NMR (500 MHz, DMSO, ppm): δ 0.87 (t, J = 7.0 Hz, 12H, CH_3), 1.21-1.39 (m, 80H, CH_2), 1.64-1.67 (m, 4H, CH_2), 2.25-2.44 (m, 12H, NCH_2), 3.44 (br, 4H, C*H*OH), 3.79 (br, 2H, COC*H*), 4.21 (d, *J* = 3.0 Hz, 2H, CHO*H*), 4.27 (d, *J* = 3.0 Hz, 2H, CHO*H*), 8.11 (br, 2H, CON*H*).

Nucleic acids. siRNAs were produced by Alnylam, which were characterized by electrospray mass spectrometry and anion exchange HPLC. The sequences for the sense and antisense strands are as follows (*in vitro* IC_{50}: FVII: ~ 10 pM; CD45: ~90 pM; Tie2: ~25 pM; PTEN: < 1 pM):
siLuc sense: 5'-AAcGcuGGGcGuuAAucAAdTdT-3', antisense: 5'-UUGAUuAACGCCcAGCGUUdTsdT-3';
siFVII sense: 5'-GGAUfCfAUfCfUfCfAAGUfCfUfUfACfdTsdT-3', antisense: 5'-GUfAAGACfUfUfGAGAUfGAUfCfCfdTsdT-3';
siGFP-Alexa647 sense: 5'-AcAuGAAGcAGcACGACuUdTsdT-3', antisense: 5'-AAGUCGUGCUGCUUCAUGUdTdT-3'-Alexa647;

siCD45 sense: 5'-TTCCTCCATGCTTGGCCAGTATTC-3', antisense: 5'-TCCCAGATCATCCTCCAGAAGTCA-3'
siPten sense: 5'-GAuGAuGuuuGAAAcuAuudTsdT-3', antisense: 5'-AAuAGUUUcAAAcAUcAUCdTsdT-3'
siTie2 sense: 5'-GAAGAuGcAGuGAuuuAcAdTsdT-3', antisense: 5'-UGuAAAUcACUGcAUCUUCdTsdT-3'
siTTR sense: 5'-GuAAccAAGAGuAuuccAudTdT-3', antisense: 5'-AUGGAAuACUCUUGGUuACdTdT-3';
2'-OMe modified nucleotides are in lower case. 2'-fluoro modified nucleotides are denoted by "f," and phosphorothioate linkages are represented by "s." siRNAs were generated by annealing equimolar amounts of complementary sense and antisense strands.

Assembling of LPNs. Lipoamino acid, lipopeptide, or lipopolypeptide derivatives were formulated with cholesterol, DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine), mPEG2000-DMG [polyethylene glycol modified lipid (R)-3-[(ω-methoxy-PEG2000-carbamoyl)]-1,2-di-O-tetradecyl-sn-glyceride, synthesized by Alnylam], and siRNA via a microfluidic based mixing device. Formulations were then dialyzed against PBS in 3,500 MWCO dialysis cassettes overnight. Particles were characterized with a modified Ribogreen assay for siRNA entrapment and dynamic light scattering (ZetaPALS, Brookhaven Instruments) for mean particle diameter. cKK-E12 LPNs were made from cholesterol, DSPC, and mPEG2000-DMG using a similar method at a molar ratio of 50:10:38.5:1.5. This formulation afforded a mean particle diameter of ~70 nm with approximately 65% siRNA entrapment efficiency.

***In Vivo* Factor VII Silencing in Mice & Rats.** All procedures used in animal studies conducted at MIT and Alnylam were approved by the Institutional Animal Care and Use Committee (IACUC) and were also consistent with local, state and federal regulations as applicable. C57BL/6 mice (Charles River Labs, 6 to 8 weeks old, 18-22 grams, and 10 ul/g), Sprague-Dawley rats (Charles River Labs, 8 weeks old, 170-200 grams, and 5 ul/g), *apoE*$^{-/-}$ mice (Jackson Laboratories, C57BL/6 background, 6 to 8 weeks old, 18-22 grams, and 10 ul/g), and *LDLR*$^{-/-}$ mice (Jackson Laboratories, C57BL/6 background, 6 to 8 weeks old, 18-22 grams, and 10 ul/g) were administered intravenously via tail vein injection for siRNA silencing experiments. After 24 or 48 h, animals were anaesthetized by isofluorane inhalation for blood sample collection by retroorbital eye bleed using serum separation tubes (Falcon tubes, Becton Dickinson). Protein levels of Factor VII were calculated by chromogenic assay (Biophen FVII, Aniara Corporation) with a standard curve obtained from control mice.

Gene Silencing in Endothelial Cells in Mice. Endothelial and hepatocyte silencing were examined 48 h after injection. Mice were sacrificed by CO2 asphyxiation, and lung, liver, heart, and kidney tissues were harvested and immediately frozen in liquid nitrogen. Frozen tissues were pulverized, and tissue lysates were prepared in Tissue and Cell Lysis Buffer (Epicentre) supplemented with 0.5 mg/ml Proteinase K (Epicentre). Factor VII silencing was evaluated in liver lysates, while Tie2 silencing was evaluated in lysates from all 4 tissues collected using a branched DNA assay (QuantiGene 2.0 Reagent System, Affymetrix). A standard curve for each tissue and target gene was constructed using samples from PBS-treated mice, and the relative

silencing in treated groups was determined by measuring each individual target gene/GAPDH level and normalizing to the corresponding ratio for PBS-treated mice controls.

Gene Silencing in Immune Cells in Mice. Mice were injected with 1 mg/kg of cKK-E12 LNP-siRNA, with siRNA specific for CD45 or Luciferase. Three days after injection, leukocytes were isolated from bone marrow, peritoneal cavity, and spleen to analyze surface CD45 expression in cells from animals injected with CD45 versus control Luc siRNA. Flow cytometric analysis was performed following standard previously described procedures. Antibodies specific for mouse CD19, CD11b, GR-1, TCRb (eBioscience, San Diego, CA) were used. Antibodies were conjugated to fluorescein isothiocyanate, phycoerythrin, allophycocyanin (APC), phycoerythrin-Cy7, or biotin. Biotinylated antibodies were detected with streptavidin conjugated to fluorescein isothiocyanate, phycoerythrin, or APC. Stained cells were analyzed using BD LSRII (BD Biosciences– Pharmingen). Flow cytometric data analysis was done using FlowJo software (Tree Star, Ashland, OR).

Cellular Internalization Assays. HeLa cells were seeded at 1.5 x 10^4 cells per well in black 96-well plates (Greiner Bio-one). Cells were incubated with 50ng cKK-E12 BLNPs in the presence of ApoE (1µg/well) for 3 hrs. Incubation time of labeled markers for different endocytic pathways is based on their internalization rate. For example, Oregon Green Dextran (300 µg/ml marker for macropinocytosis) was co-incubated for 3 hrs, while Alexa 488-Transferrin (1µg/ml, marker for clathrin mediated endocytosis) and Alexa 488-Cholera Toxin B (10 µg/ml, marker for caveolae mediated endocytosis) were co-incubated for 15 and 30 mins, respectively, with the BLNPs. Cells were washed, fixed, and counterstained in OPTI-MEM containing Hoescht (2 µg/ml) for nuclei identification. Triple-stained live cell imaging was performed with an automated spinning disk confocal microscope (OPERA, Perkin Elmer) with a 40X objective. The same defined pattern of 20 fields from each well was acquired to eliminate bias and provide a statistically significant number of cells for analysis.

***In Vitro* siRNA Transfection Assay and Microscopy.** Effects of apolipoproteins were evaluated through an *in vitro* siRNA transfection assay in HeLa cells as previously reported. HeLa cells, stably expressing firefly luciferase and Renilla luciferase were seeded in an opaque white 96-well plate (Corning-Costar) overnight. Cells were transfected by cKK-E12 formulated 50 ng of firefly-specific siLuc in quadruplicate. Apolipoproteins (Fitzgerald Industries) were incubated with cKK-E12 formulations for 5 mins before adding to cells. After 24 h incubation at 37 ^{o}C, 5% CO_2, cells were analyzed for luciferase expression using Dual-Glo assay kits (Promega). For visualization of cell uptake, cKK-E12 was formulated with an Alexa-Fluor 647–labeled siRNA and incubated with Hela cells for 3 h. Cells were then fixed in 4% paraformaldehyde, permeabilized with 0.1% saponin and stained with Hoescht. All images were acquired using an Opera spinning disc confocal system (Perkin Elmer), and the data was analyzed using Acapella Software (Perkin Elmer).

***In Vivo* Delivery of siRNA in Rats and its Tolerability.** To evaluate whether the silencing activity in mice could be translated to other animal species, we further studied the delivery efficiency of cKK-E12 in rats. As shown in figure S5, cKK-E12 LPNs showed significant dose-dependent silencing with an ED_{50} lower than 0.01 mg/kg. To examine the potential for toxicity with cKK-E12, we performed a single-dose tolerability study in rats (0.3 and 1 mg/kg). There was no significant alteration of clinical signs and body weight in treated groups compared with

control group. cKK-E12 LPNs was well tolerated with no clinically significant changes in key serum chemistry parameters at doses up to 1 mg/kg (Table S2).

Silencing Effect of cKK-E12 LPNs in Nonhuman Primates. All procedures using Cynomolgus monkeys (3.4 to 3.8 years old, 2.4 to 3.0 kg, and 5 mL/kg) were conducted by a certified contract research organization using protocols consistent with local, state and federal regulations as applicable and approved by the Institutional Animal Care and Use Committee (IACUC). cKK-E12 was formulated with siRNA specific to transthyretin (TTR) through 15-min intravenous infusions (5 mL/kg) via the cephalic vein in cynomolgus monkeys (three per group) at a dose of 0.3 mg/kg. TTR mRNA levels were calculated relative to GAPDH mRNA levels using a branched DNA assay (QuantiGene Assay, Affymetrix, CA).

Table S1. A summary of the calculated and observed m/z ratios in the LC-MS spectra of amino acid-based lipid derivatives, siRNA entrapment, and a representative structure of K-E12.

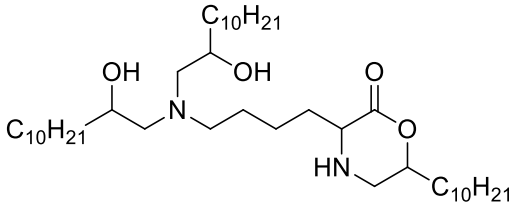
Compd	Chemical formula		Calcd.	Observed	Tail #	Entrapment (%)
A-E12	C27H54NO3+		440.4098	440.4336	2	53
C-E12	C27H54NO3S+		472.3819	472.4303	2	0
D-E12	C28H54NO5+		484.3997	484.4327	2	0
E-E12	C29H56NO5+		498.4153	498.4117	2	0
F-E12	C33H58NO3+		516.4411	516.4332	2	0
G-E12	C26H52NO3+		426.3942	426.3772	2	0
H-E12	C42H80N3O4+		690.6143	690.6016	3	0
I-E12	C30H60NO3+		482.4568	482.4461	2	6
K-E12	C42H85N2O4+		681.6504	681.6009	3	56
L-E12	C30H60NO3+		482.4568	482.4771	2	0
M-E12	C29H58NO3S+		500.4132	500.4471	2	0
N-E12	C40H79N2O5+		667.5984	667.5894	3	0
P-E12	C17H32NO2+		282.2428	282.2585	1	0
Q-E12	C29H57N2O4+		497.4313	497.4268	2	0
R-E12	C54H109N4O5+		893.8392	893.8400	4	50
S-E12	C27H54NO4+		456.4047	456.3891	2	0
T-E12	C28H56NO4+		470.4204	470.4186	2	0
V-E12	C29H58NO3+		468.4411	468.4259	2	0
W-E12	C35H59N2O3+		555.4520	555.4510	2	10
Y-E12	C33H58NO4+		532.436	532.4149	2	10
cKG-E12	C32H64N3O4+		554.4891	554.4852	2	50^{P}
cKT-E12	C34H68N3O5+		598.5153	598.5179	2	31
cYK-E12	C39H70N3O5+		660.5310	660.5350	2	15
cLK-E12	C36H72N3O4+		610.5517	610.5556	2	64^{P}
cDK-E12*	C36H70N3O6+		640.5259	640.5316	2	63
cMK-E12	C35H70N3O4S+		628.5082	628.5072	2	73

cKV-E12	C35H70N3O4+		596.5361	596.5330	2	53[P]
cAK-E12	C33H66N3O4+		568.5048	568.4992	2	72
cCK-E12	33H66N3O4S+		600.4769	600.6143	2	ND
cQK-E12	C35H69N4O5+		625.5262	625.4733	2	44
cPK-E12	C35H68N3O4+		594.5204	594.5169	2	64
cFK-E12	C39H70N3O4+		644.5361	644.5301	2	48[P]
cWK-E12	C41H71N4O4+		683.5470	683.5367	2	49
cEK-E12	C35H68N3O6+		626.5103	626.5053	2	4[P]
cIK-E12	C36H72N3O4+		610.5517	610.5501	2	49
cSK-E12	C33H66N3O5+		584.4997	584.5029	2	18
cKK-E10	C52H105N4O6+		881.8029	881.8042	4	31
cKK-E12	C60H121N4O6+		993.9281	993.9224	4	65
cKK-E14	C68H137N4O6+		1106.0533	1106.0709	4	50
cKK-E16	C76H153N4O6+		1218.1785	1218.2002	4	52
A-A12	C27H56NO2+		426.4306	426.4244	2	0
C-A12	C27H56NO2S+		458.4026	458.3857	2	ND
D-A12	C28H56NO4+		470.4204	470.4188	2	ND
E-A12	C29H58NO4+		484.4360	484.4319	2	0
F-A12	C33H60NO2+		502.4619	502.4560	2	0
G-A12	C26H54NO2+		412.4149	412.4107	2	0
H-A12	C30H58N3O2+		492.4524	492.4503	2	71
I-A12	C30H62NO2+		468.4775	468.4714	2	0
K-A12	C54H111N2O2+		819.8640	819.8657	4	15
L-A12	C30H62NO2+		468.4775	468.4752	2	0
M-A12	C29H60NO2S+		486.4339	486.4318	2	0
N-A12	C28H57N2O3+		469.4364	469.4328	2	0
P-A12	C17H34NO2+		284.2584	284.2512	1	0
Q-A12	C29H59N2O3+		483.4520	483.4543	2	0
R-A12	C42H87N4O2+		679.6824	679.6783	3	62
S-A12	C27H56NO3+		442.4255	442.4225	2	0
T-A12	C28H58NO3+		456.4411	456.4398	2	0
V-A12	C29H60NO2+		454.4619	454.4544	2	0
W-A12	C35H61N2O2+		541.4728	541.4724	2	0
Y-A12	C33H60NO3+		518.4568	518.4543	2	ND
KK-A12	C84H171N4O3+		1284.3346	1284.3458	6	51
KKK-A12	C114H231N6O4+		1748.8051	1748.8340	8	ND
cKK-A12	C60H121N4O2+		929.9484	929.9445	4	69
A-O12	C18H36NO4+		330.2639	330.2582	1	ND
C-O12	C33H64NO6S+		602.4449	602.4426	2	ND

D-O12	C19H36NO6+		374.2537	374.2492	1	ND
E-O12	C20H38NO6+		388.2694	388.2672	1	ND
F-O12	C24H40NO4+		406.2952	406.2896	1	ND
G-O12	C17H34NO4+		556.4572	556.4551	2	12
H-O12	C36H66N3O6+		636.4946	636.4969	2	54
I-O12	C21H42NO4+		372.3108	372.3054	1	ND
K-O12	C66H127N2O10+		1107.9485	1107.9417	4	61
L-O12	C21H42NO4+		372.3108	372.3052	1	ND
M-O12	C20H40NO4S+		390.2673	390.2628	1	ND
N-O12	C19H37N2O5+		373.2697	373.2668	1	ND
P-O12	C20H38NO4+		356.2795	356.2779	1	ND
Q-O12	C20H39N2O5+		387.2853	387.2831	1	ND
R-O12	C21H43N4O4+		415.3279	415.3235	1	ND
S-O12	C18H36NO5+		346.2588	346.2521	1	ND
T-O12	C19H38NO5+		360.2744	360.2733	1	ND
V-O12	C20H40NO4+		358.2952	358.2905	1	ND
W-O12	C26H41N2O4+		445.3061	445.3010	1	ND
Y-O12	C24H40NO5+		422.2901	422.2868	1	ND
KK-O12	C87H167N4O13+		1476.2524	1476.2533	5	ND
KKK-O12	C123H235N6O18+		2084.7652	2084.7650	7	ND
cKK-O12	C72H137N4O10+		1218.0329	1218.0880	4	75

*: formation of ethyl ester. ND: compounds were not dissolved in the formulation solution. [P]: formulations were precipitated after dialysis. Compounds derived from poly-L-lysine are not included. Our screening criteria were as follows: 1. Compounds were not formulated if they were unable to be dissolved in formulation solution (10% citrate buffer in ethanol). 2. Materials were considered to be unstable, if solid precipitated from the formulation solution after dialysis. These materials were not evaluated in our bioassays. siRNA entrapment was evaluated using a Ribogreen assay reported previously [1].

Structure of K-E12:



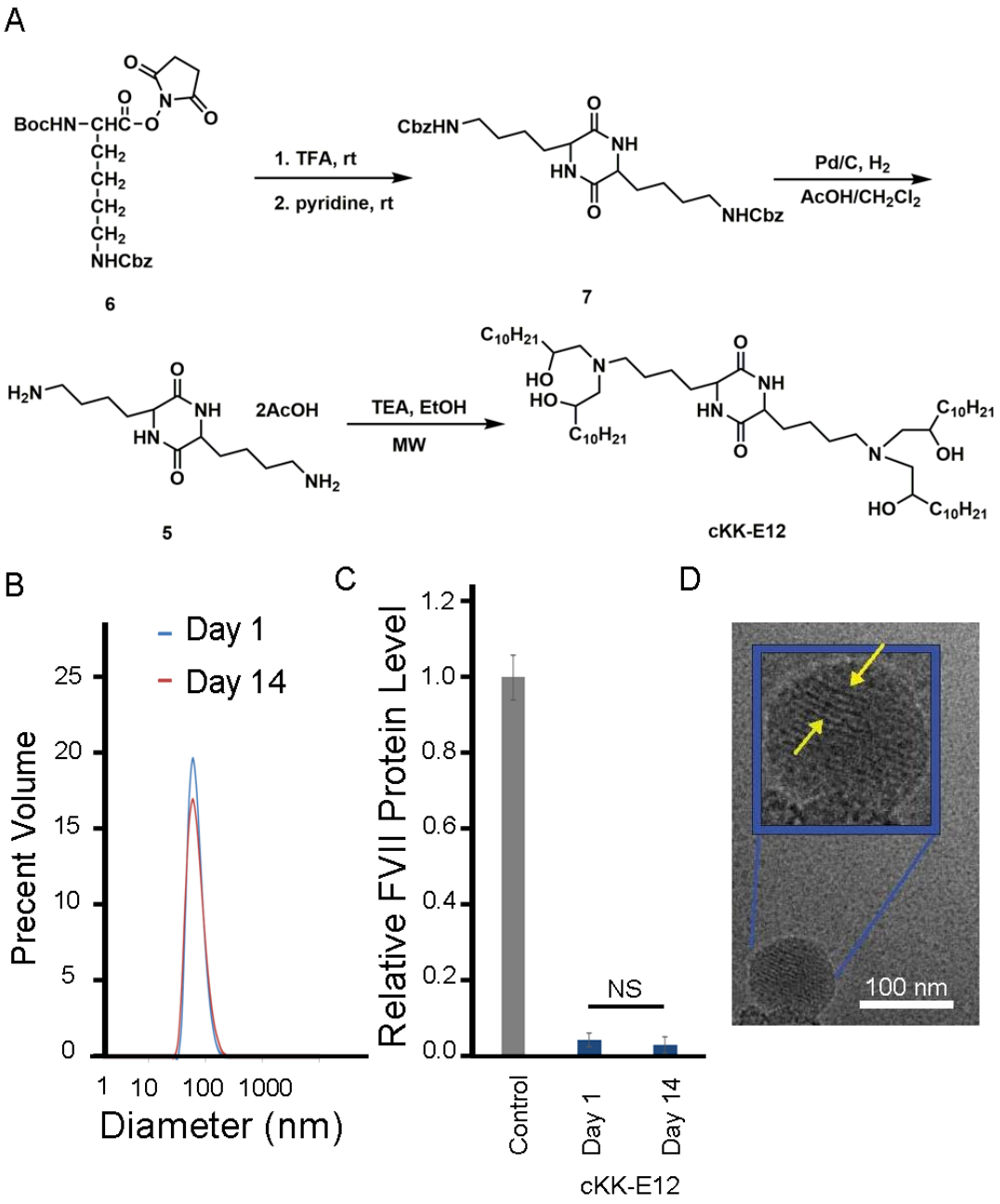


Fig. S1. Synthesis and characterization of cKK-E12 LPNs. (*A*) An alternative synthetic route to cKK-E12. (*B*) Particle size of cKK-E12 LPNs measured by dynamic light scattering (DLS) at day 1 (69.3 nm, PDI= 0.138) and 14 (70.8 nm, PDI= 0.098). (*C*) FVII silencing of cKK-E12 LPNs at day 1 and 14. (*D*) a cryo-TEM image of cKK-E12 LPNs with a textured interior.

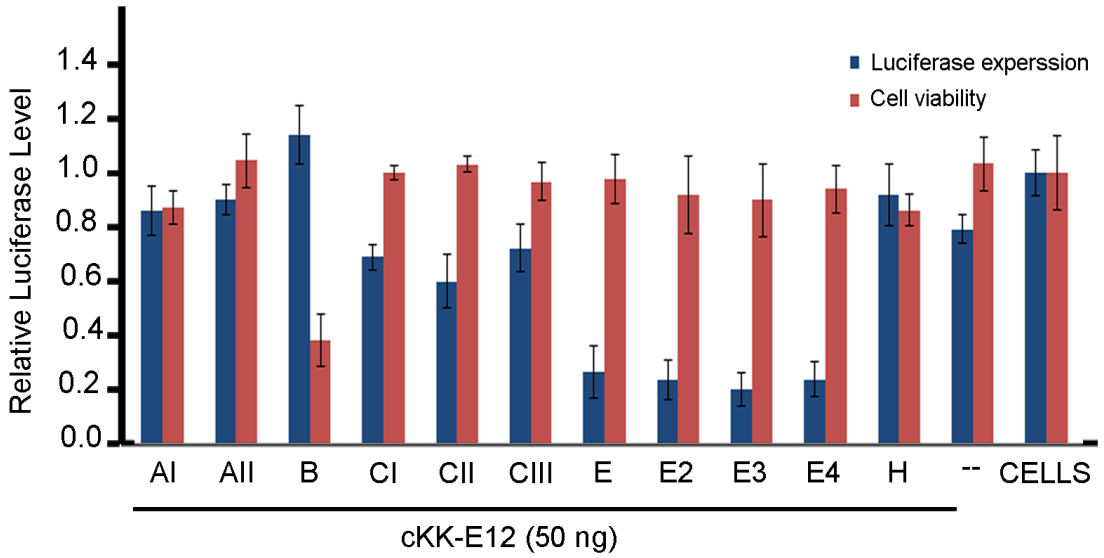


Fig. S2. Silencing effects of apolipoproteins on cKK-E12 in HeLa cells. apolipoproteins including ApoA-I (recombinant Human ApoA-I protein), ApoA-II (native Human ApoA-II protein), ApoB (native Human ApoB protein), ApoC-I (native Human ApoC-I protein), ApoC-II (native Human ApoC-II protein), ApoC-III (native Human ApoC-III protein), ApoE (native Human ApoE protein), ApoE2 (recombinant Human ApoE2 protein), ApoE3 (recombinant Human ApoE3 protein), ApoE4 (recombinant Human ApoE4 protein), ApoH (native Human ApoH protein).

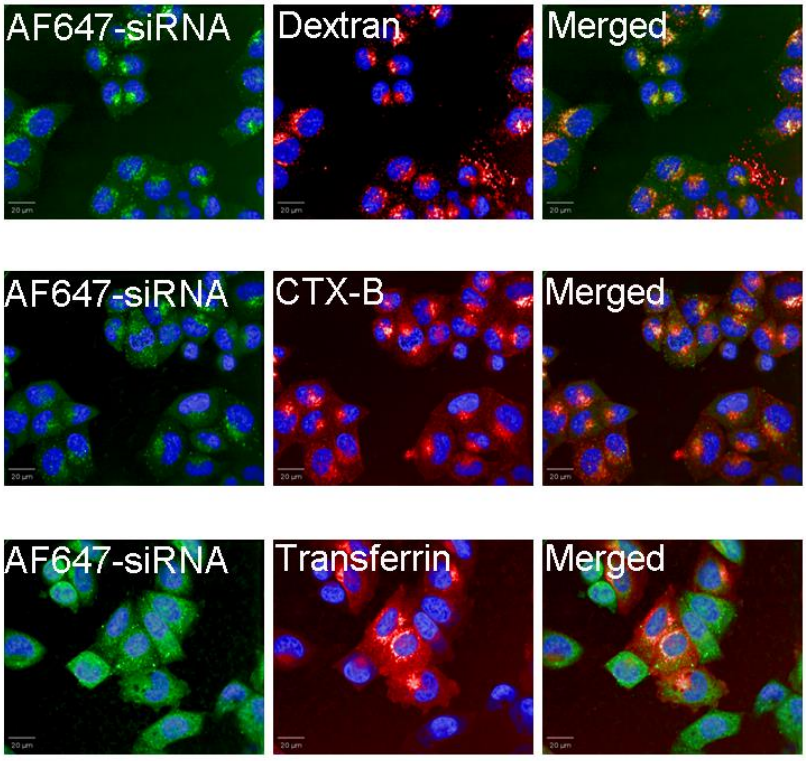


Fig. S3. cKK-E12-siRNA LNPs cellular internalization. Co-localization studies of labled-siRNA BLNPs with labeled makers for different endocytic pathways including dextran, Cholera toxin B (CTX-B), or transferrin. The labeled BLNPs co-localized with dextran (co-localization percentage: 17.7 ± 0.2) but significantly ($P<0.05$) less with transferrin (co-localization percentage: 6.3 ± 0.8) or Cholera toxin B (co-localization percentage: 10.2 ± 0.3). n= 30 images. Scale bar: 20 μm.

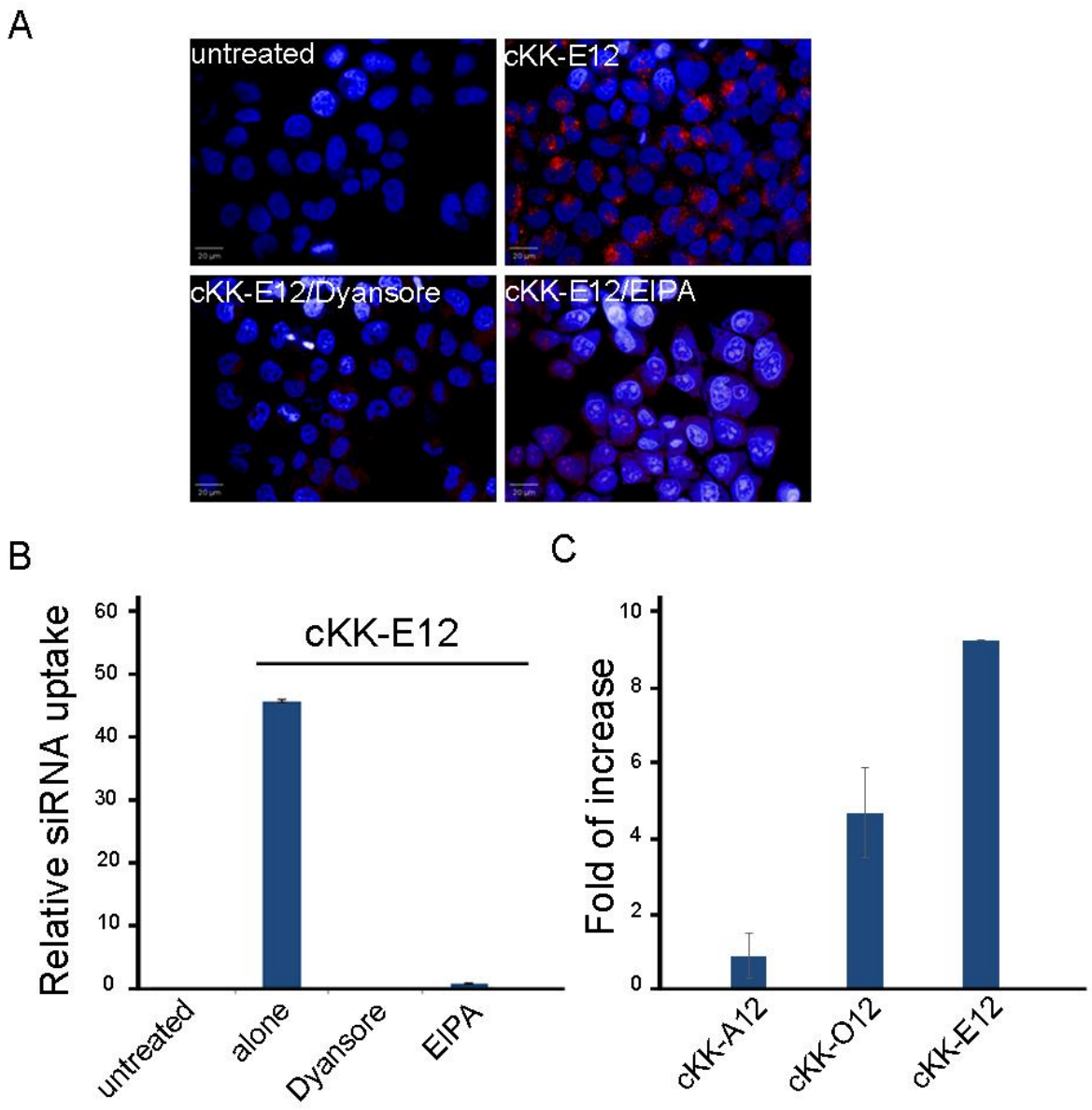


Fig. S4. cKK-E12-siRNA LNPs cellular internalization through dyanmin-dependent macropinocytosis. (*A*) Cell uptake studies of labled-siRNA BLNPs in the presence of apoE with dynasore (dynamin inhibitor) and EIPA (macropinocytosis inhibitor). (*B*) Quantification of cell uptake. Scale bar: 20 μm. (*C*) Impacts of apoE on the cellular uptake of cKK-A12, cKK-O12, and cKK-E12 LPNs.

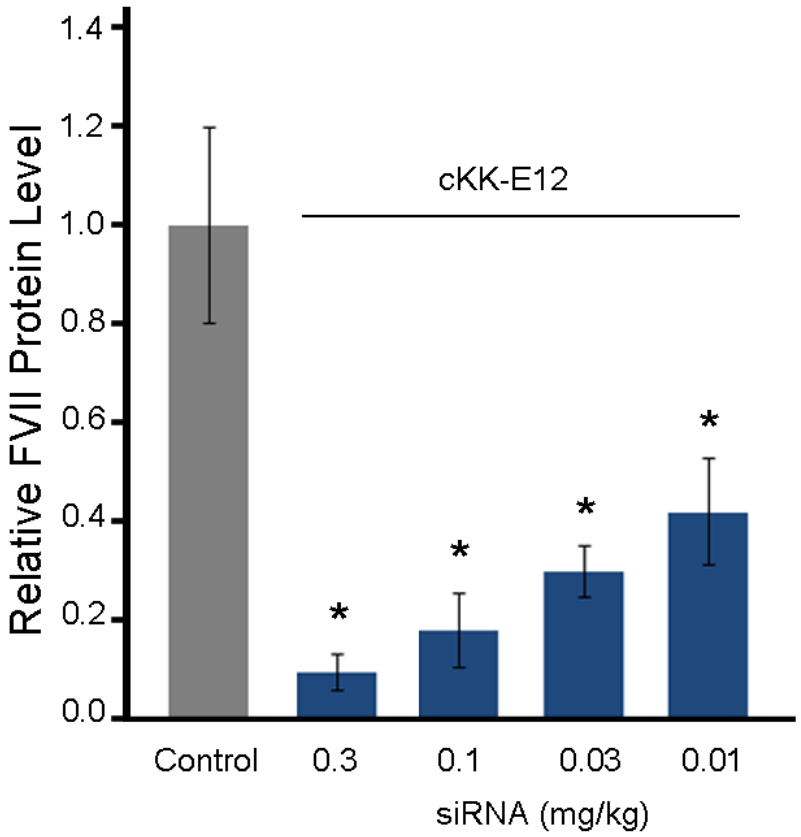


Fig. S5. Silencing effects of cKK-E12 LPNs in rats. Sprague-Dawley rats were administered FVII-formulated cKK-E12 via intravenous infusions. Data points represent group mean ± s.d. (n= 3; *, P < 0.05; t-test, double-tailed).

Table S2. **Clinical chemistry for cKK-E12–treated rats**

siRNA (mg/kg)	time	ALT (U/L)	AST (U/L)	TBILI (mg/dL)	TBA (umol/L)	CHOL (mg/dL)	CREAT (mg/dL)	BUN (mg/dL)
Control		54±12	99±11	0.18±0.03	56±34	92±15	0.32±0.05	13±2
0.3	24 h	55±7	114±27	0.17±0.02	40±28	91±10	0.34±0.04	16±1
1		59±8	101±12	0.16±0.01	45±25	84±21	0.36±0.04	16±1
Control		54±9	95±26	0.16±0.01	55±31	83±17	0.37±0.14	19±5
0.3	72 h	42±5	85±14	0.12±0.02	44±20	69±9	0.29±0.02	16±1
1		43±5	78±13	0.13±0.01	43±13	60±9	0.27±0.02	16±0.4

Sprague-Dawley rats (n = 5) received 15-min intravenous infusions of cKK-E12 LPNs at different dose levels (0.3 & 1 mg/kg). Blood samples were taken at 24 & 72 h after administration. Control, PBS; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBILI, total bilirubin; TBA, total bile acids; CHOL, cholesterol; CREAT, creatinine; BUN, blood urea nitrogen.

References

1. Heyes, J., Palmer, L., Bremner, K. & MacLachlan, I. (2005) Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids. *J Control Release* 107, 276-287.