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Supplementary Materials for

Thermostable ionizable lipid-like nanoparticle (iLAND) for RNAi treatment of hyperlipidemia

Bo Hu, Bo Li, Kun Li, Yuanyuan Liu, Chunhui Li, Lulu Zheng, Mengjie Zhang, Tongren Yang, Shuai Guo, Xiyu Dong, Tian Zhang, Qing Liu, Abid Hussain, Yuhua Weng, Ling Peng, Yongxiang Zhao, Xing-Jie Liang, Yuanyu Huang*

*Corresponding author. Email: yyhuang@bit.edu.cn

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This PDF file includes:

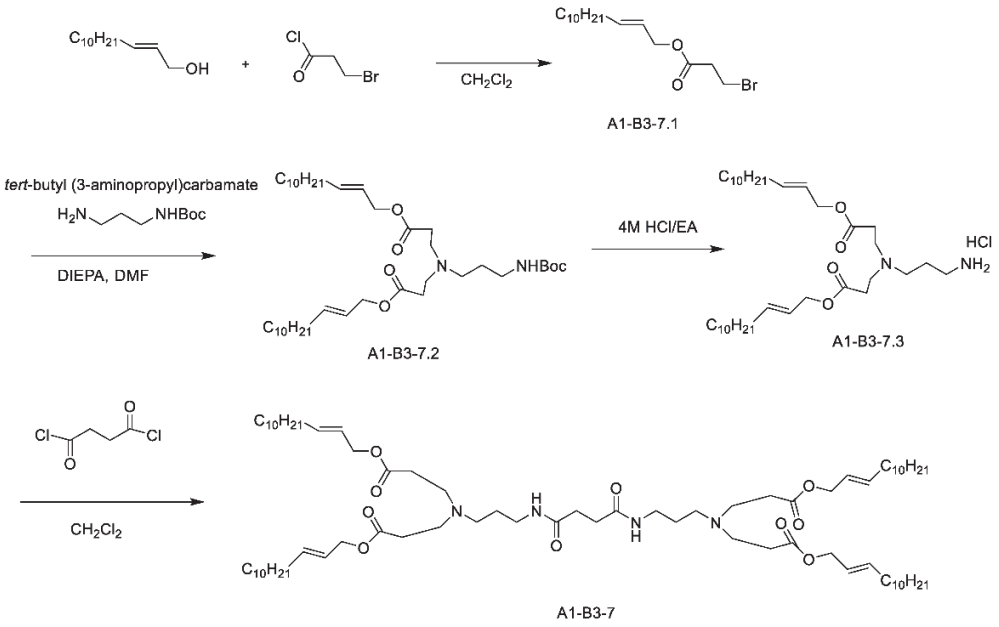

Fig. S1. The synthesis route of A1-B3-7.

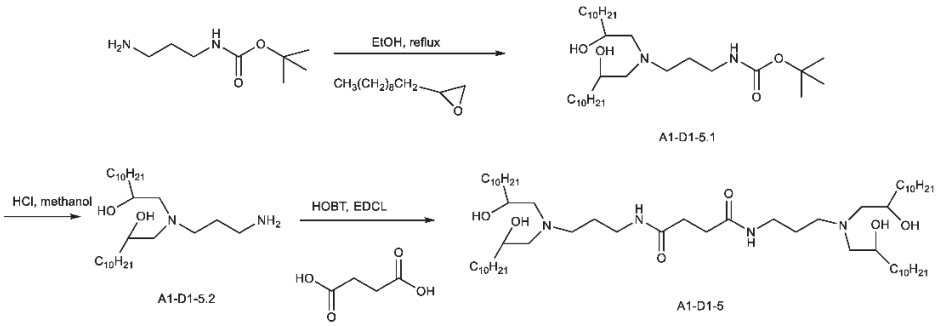

Fig. S2. The synthesis route of A1-D1-5.

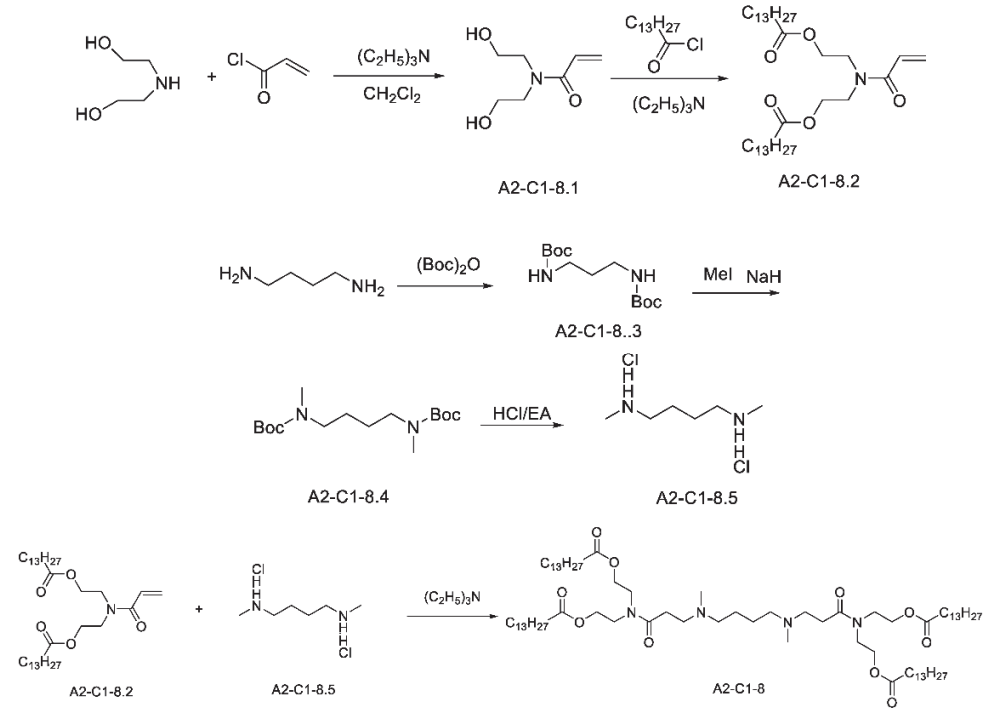

Fig. S3. The synthesis route of A2-C1-8.

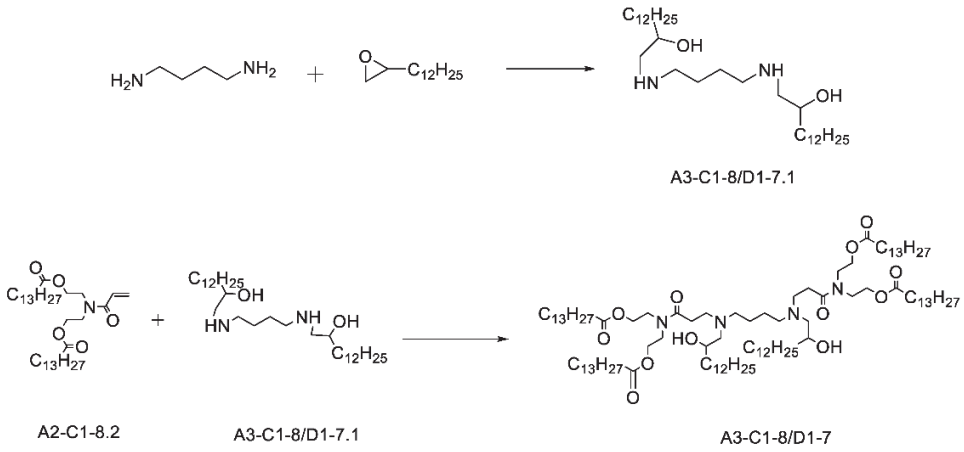

Fig. S4. The synthesis route of A3-C1-8/D1-7.

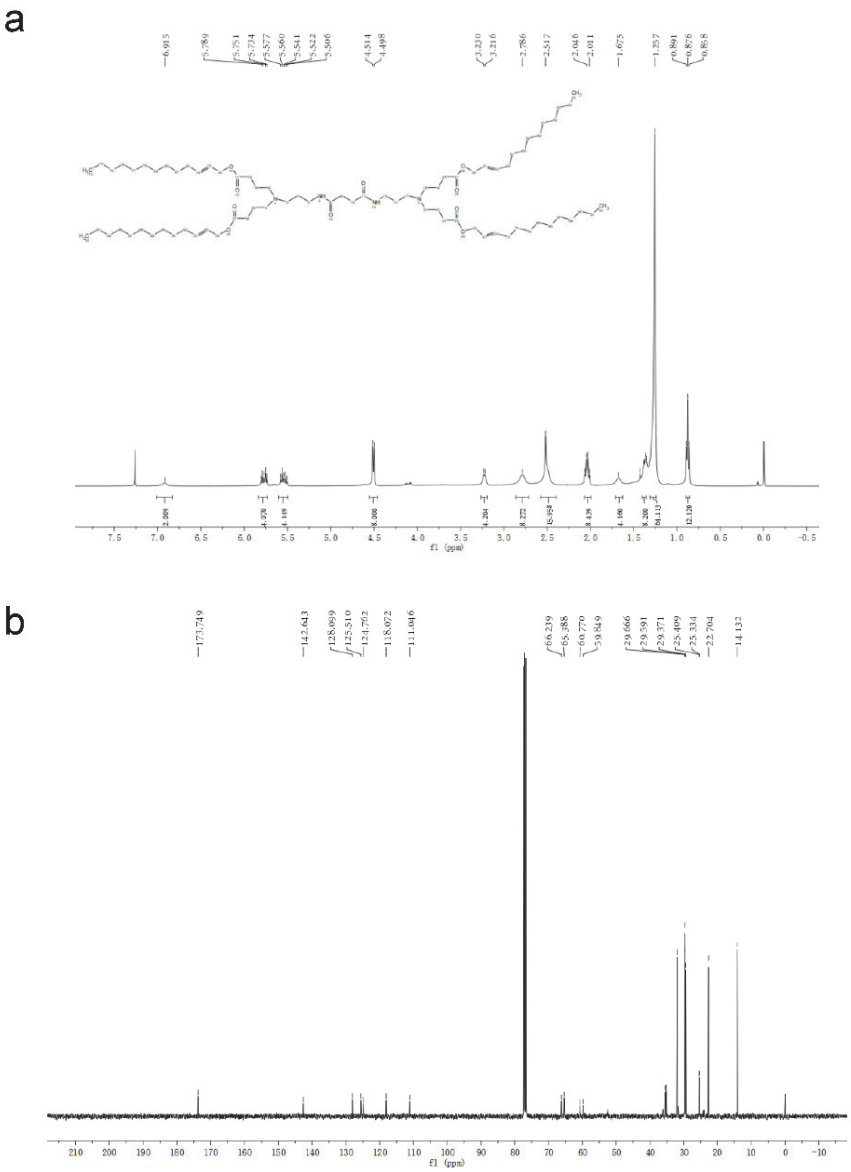

Fig. S5. Identification of A1-B3-7 using (a) ^{1}H NMR and (b) ^{13}C NMR.

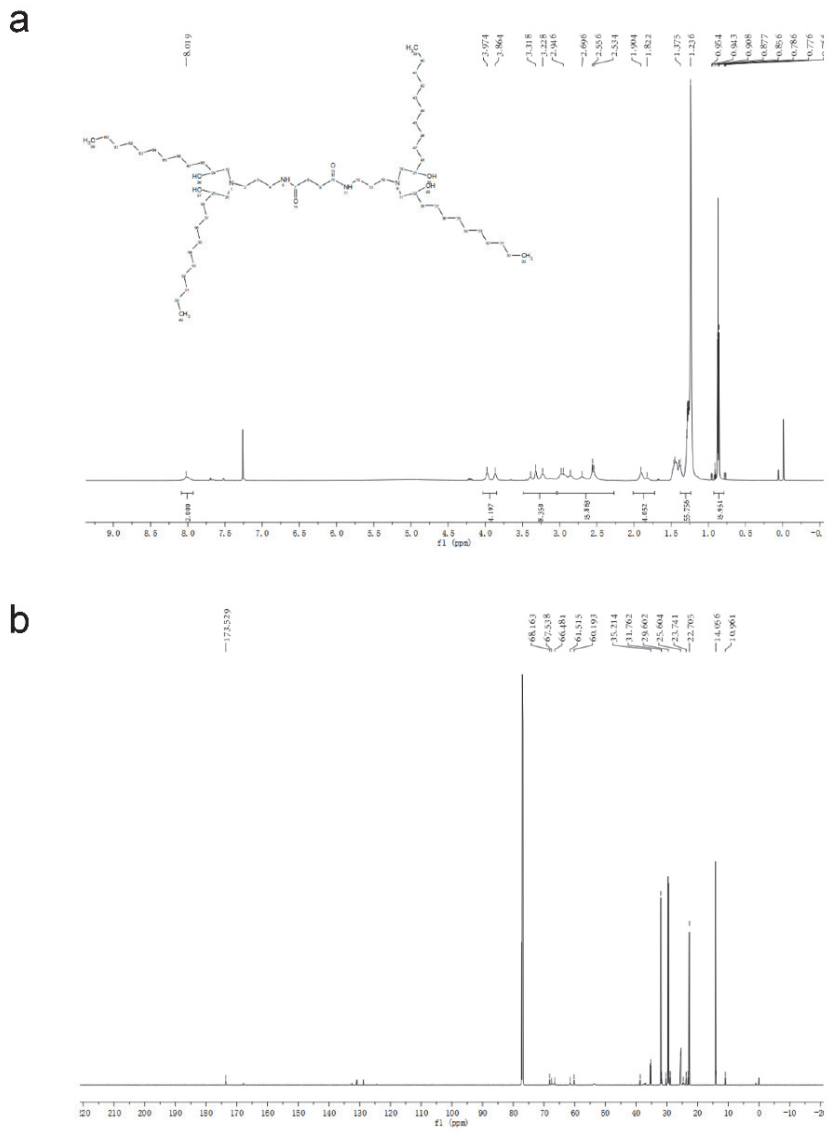

Fig. S6. Identification of A1-D1-5 using (a) ^{1}H NMR and (b) ^{13}C NMR.

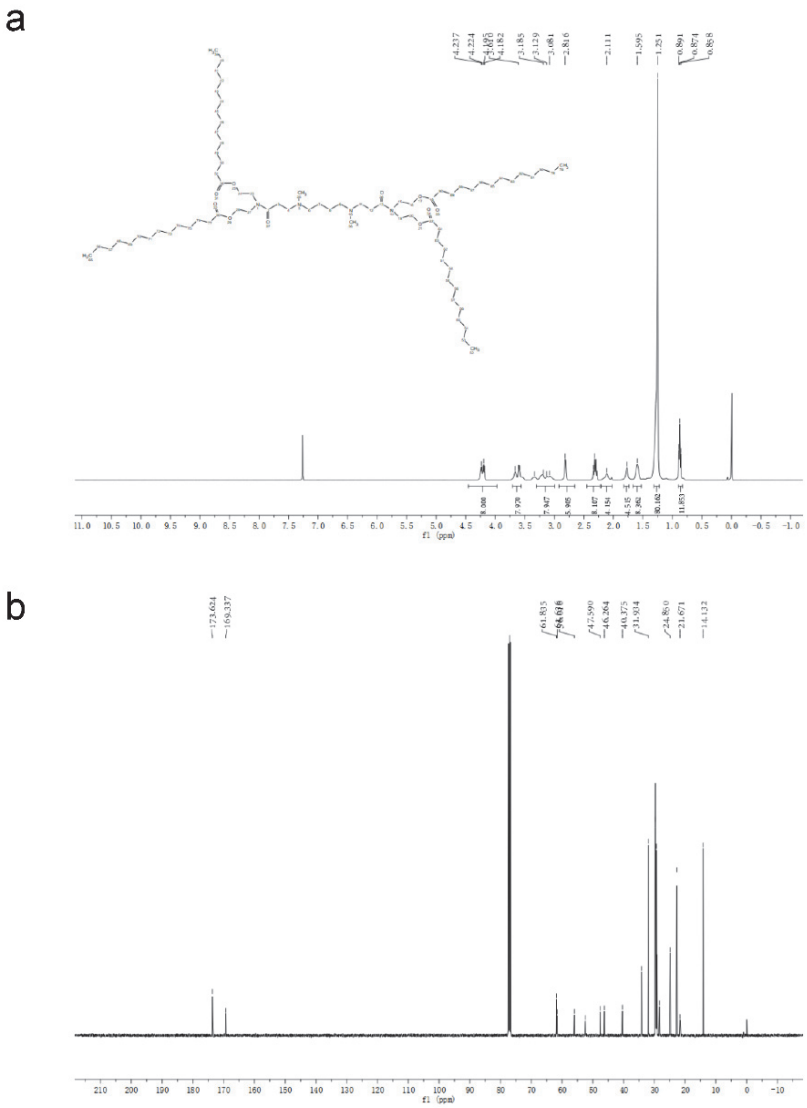

Fig. S7. Identification of A2-C1-8 using (a) ^{1}H NMR and (b) ^{13}C NMR.

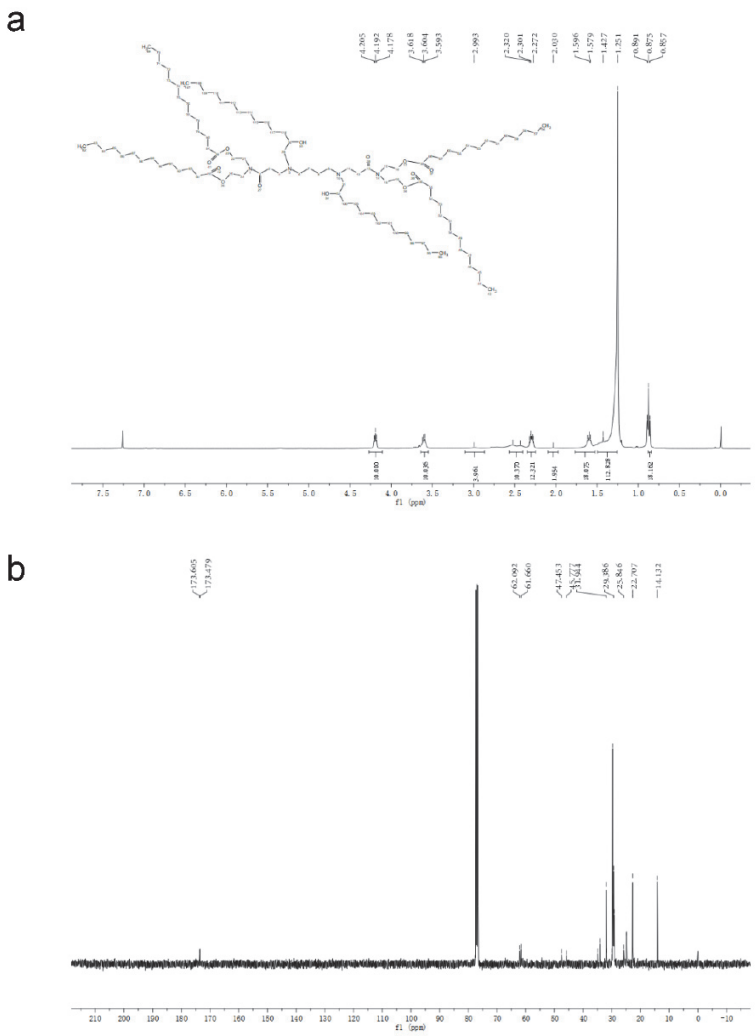

Fig. S8. Identification of A3-C1-8/D1-7 using (a) ^{1}H NMR and (b) ^{13}C NMR.

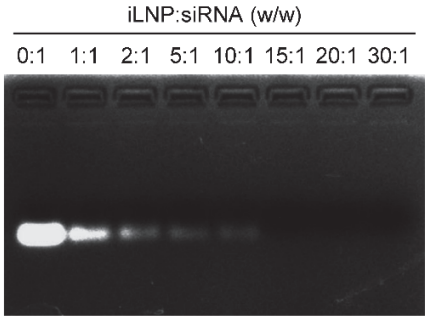

Fig. S9. Agarose gel retardation assay. The mass ratios between the iLNP and siRNA varied from 0:1 to 30:1. It was shown that siRNA could be completely entrapped by iLNP when the mass ratio was higher than 15:1.

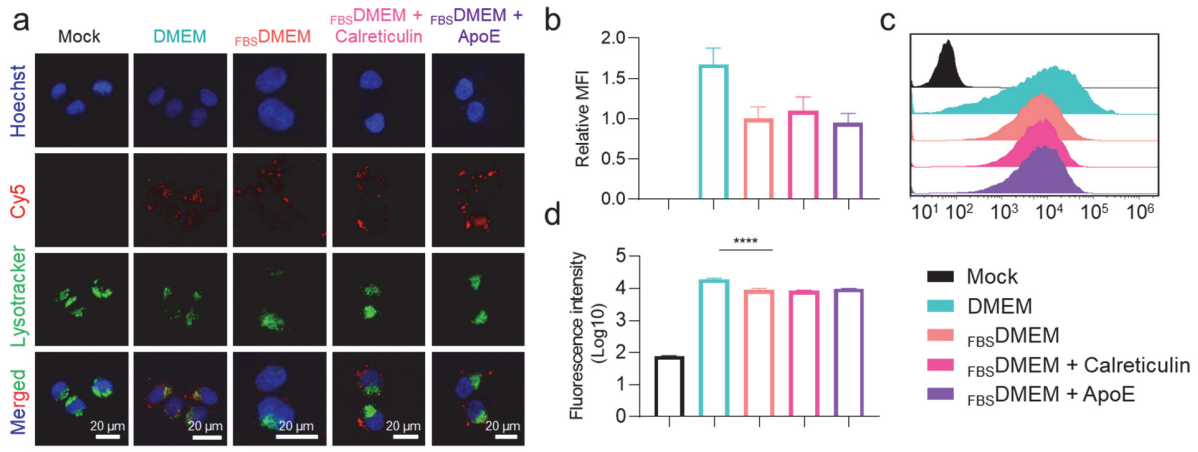

Fig. S10. Effect of ApoE and calreticulin on DOTAP LNP internalization in Hela cells. (**a**) Confocal observation of the internalization of Cy5-siRNA@DOTAP LNP. ApoE and calreticulin were used to determine if they bound with and facilitated the cellular entry of Cy5-siRNA@DOTAP LNP. (**b**) Quantitative analysis of the fluorescence intensity of siRNA as recorded in (a). (**c** and **d**) Cellular uptake of Cy5-siRNA@DOTAP LNP in Hela cells as recorded by FACS. siRNA was transfected at final concentration of 50 nM.

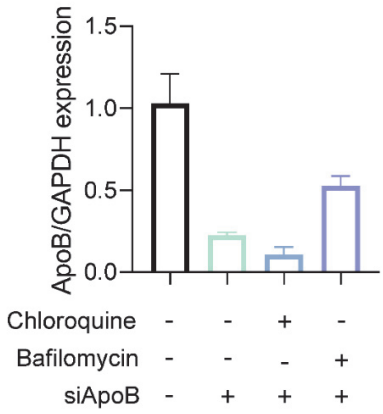

Fig. S11. Effect of chloroquine and bafilomycin A1 on siRNA silencing activity. The concentrations of chloroquine and bafilomycin A1 were 100 nM and 200 nM, respectively. siRNA was transfected at final concentration of 50 nM.

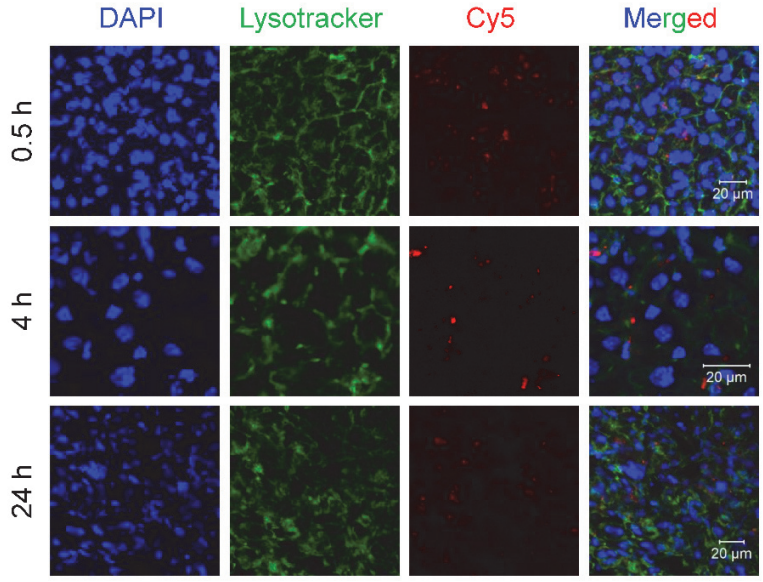

Fig. S12. Confocal observation of fluorescence signal in liver cryosections prepared with the liver tissues collected at 0.5 h, 4 h and 24 h post injection. Scale bar, 20 μm.

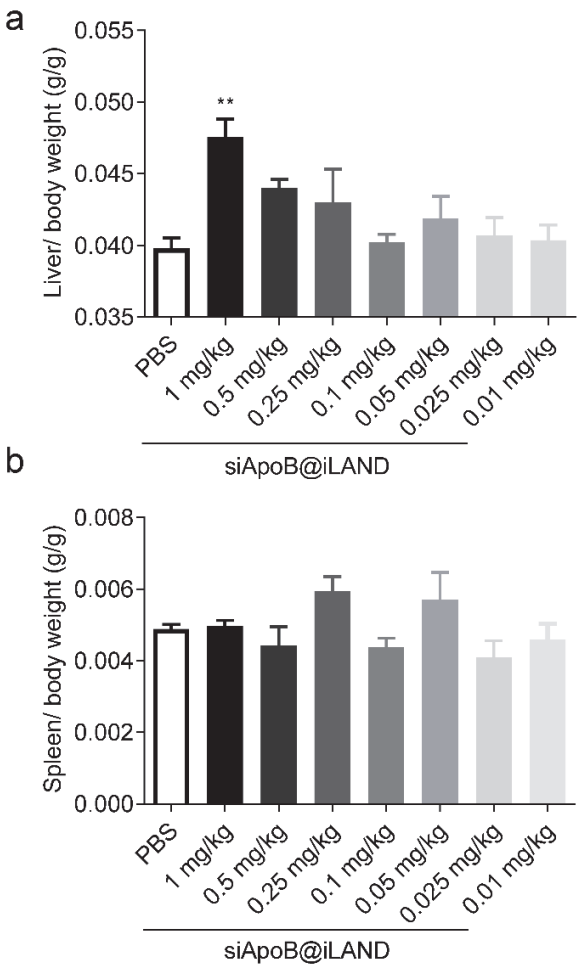

Fig. S13. Organ coefficients of the liver and spleen. The data were collected in ED_{50} test by using anti-ApoB siRNA. Each bar represents the mean ± S.E.M. **P < 0.01.

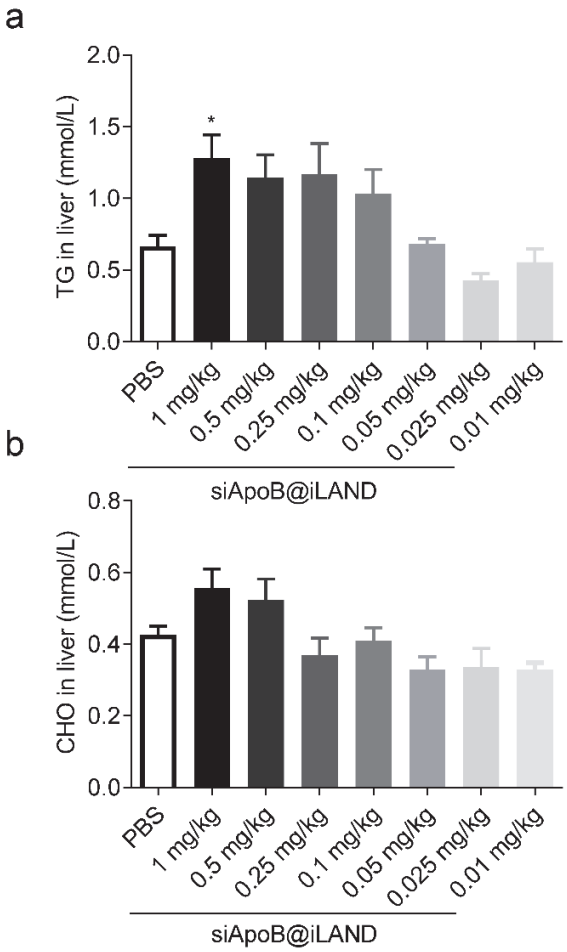

Fig. S14. Levels of TG (a) and CHO (b) in the liver in ED_{50} test by sing anti-ApoB siRNA. Each bar represents the mean ± S.E.M. *P < 0.05.

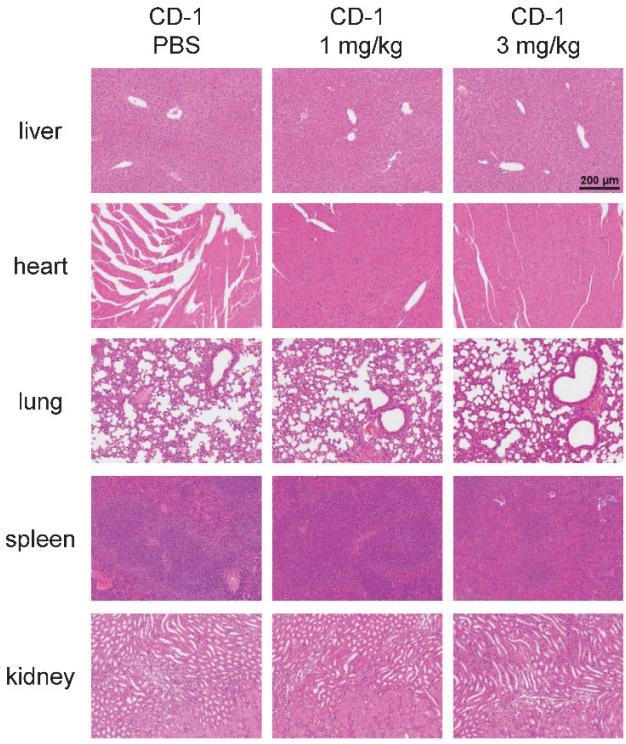

Fig. S15. H&E staining of the tissue sections prepared in the safety assay. CD-1 mice were used in this study. Scale bar, 200 μm.

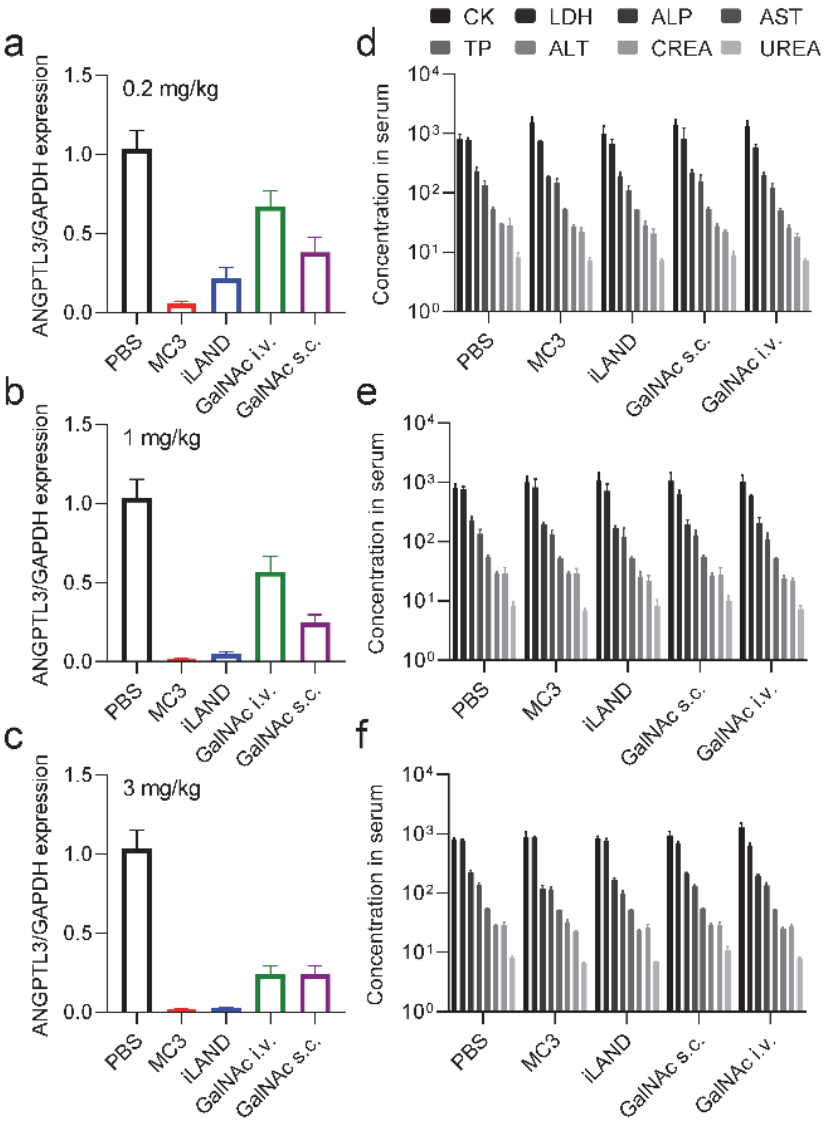

Fig. S16. Comparison of three representative siRNA delivery platforms including iLAND, Dlin-MC3-DMA LNP and GalNAc-siRNA. (a - c) Relative ANGPTL3 mRNA expression determined by RT-qPCR. Liver samples were collected from mice receiving injections of siANG@iLAND (i.v.) or siANG@MC3 (i.v.) or GalNAc-siANG (i.v. or s.c.). The doses were 0.2 mg/kg, 1 mg/kg, 3 mg/kg, respectively. (d- f) Serum biochemistry analysis. Eight parameters including creatine kinase (CK, U/L), lactate dehydrogenase (LDH, U/L), alkaline phosphatase (ALP, U/L), aspartate aminotransferase (AST, U/L), total protein (TP, g/L), alanine aminotransferase (ALT, U/L), serum creatinine (CREA, μmol/L), urea nitrogen (UREA, μmol/L) were recorded.

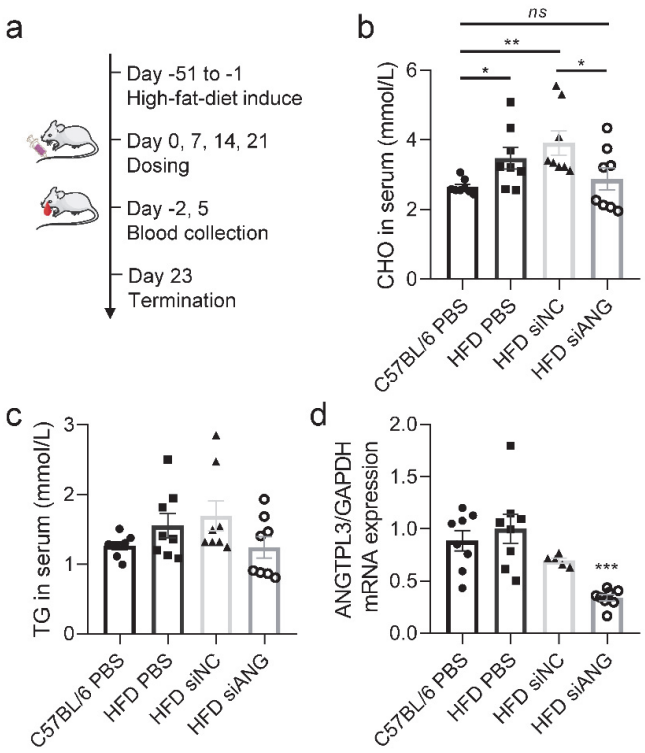

Fig. S17. Lipid-lowing effects of siANG@iLAND in high-fat diet (HFD)-fed mouse model. siNC@iLAND and siANG@iLAND were dosed at 0.25 mg/kg. (a) The treatment schedule in HFD-fed mice. The animals were fed with high-fat diet (Research Diets, D12451) for 7 weeks to establish the model, then the mice were divided into 3 groups. In parallel, there were other 8 mice were fed with standard chow diet during the same time period. All mice were received 4 doses (one dose per week) of siRNA formulation. (b) Serum CHO levels recorded at 48 h after the first dose. (c) Serum TG level recorded at 48 h after the first dose. (d) Relative ANGPTL3 mRNA expression as determined by qPCR at the end of the study. Data were shown as the mean ± S.E.M. ***P < 0.001; **P < 0.01; *P < 0.05; ns, there is no significant difference.

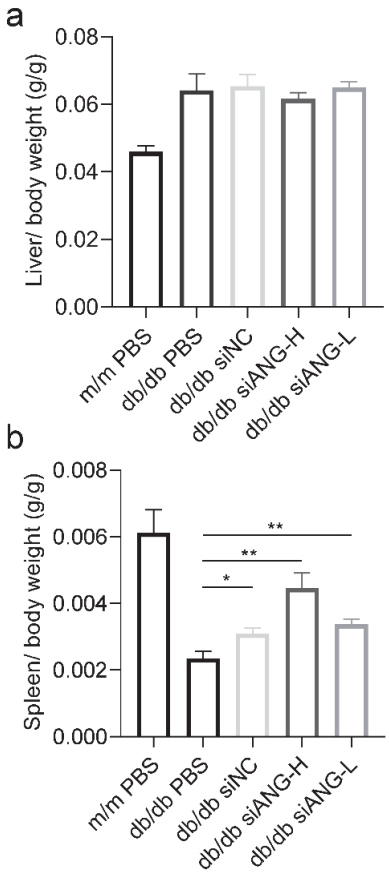

Fig. S18. Organ coefficients of the liver and spleen. Liver/body weight ratio (a) and spleen/body weight ratio (b) recorded in efficacy study by using db/db mice. Each bar represents the mean ± S.E.M. **P < 0.01, *P < 0.05.

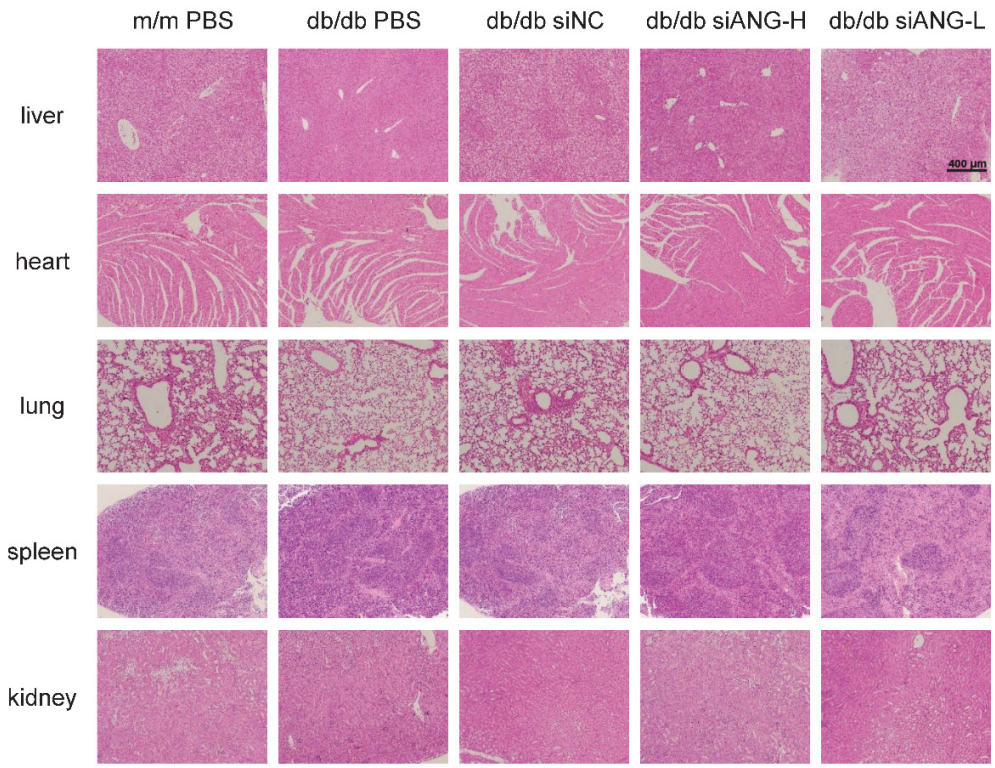

Fig. S19. H&E staining of the sections prepared with the tissues collected in the study using db/db mice. Scale bar, 200 μm.

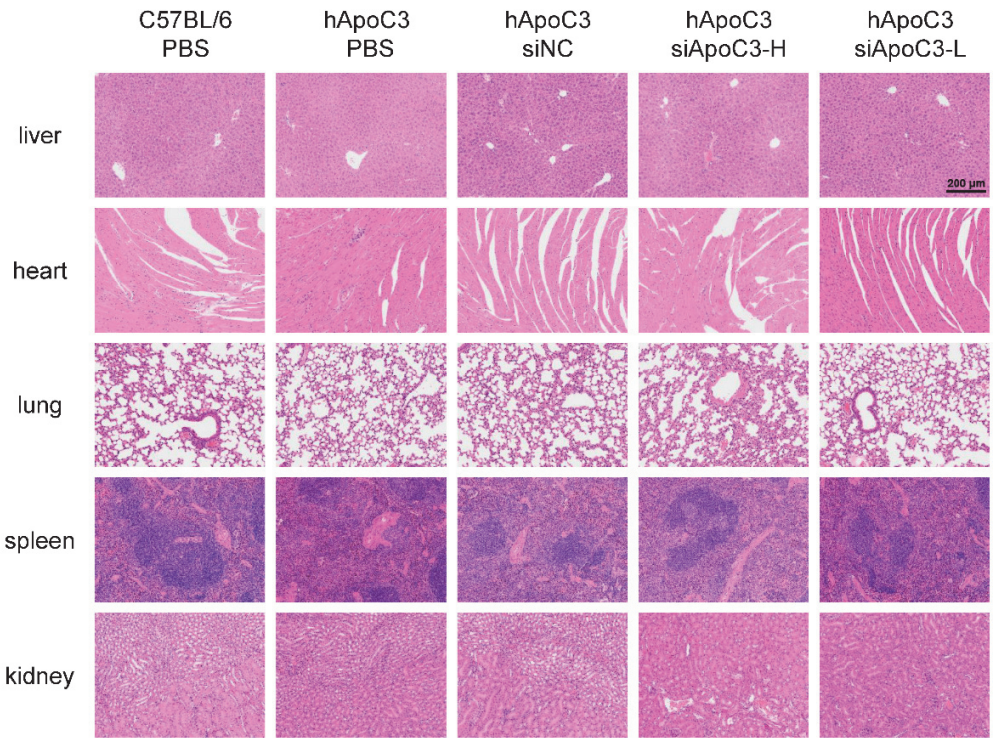

Fig. S20. H&E staining of the sections prepared with the tissues collected in the study using hApoC3-Tg mice. Scale bar, 200 μm.

Name	Molar Ratio			
	Ionizable lipid	DSPC	Cholesterol	DMG-PEG2000
1	40	8	30	0.5
2	40	12	40	1
3	40	16	50	2.5
4	40	20	60	5
5	47	8	40	2.5
6	47	12	30	5
7	47	16	60	0.5
8	47	20	50	1
9	54	8	50	5
10	54	12	60	2.5
11	54	16	30	1
12	54	20	40	0.5
13	61	8	60	1
14	61	12	50	0.5
15	61	16	40	5
16	61	20	30	2.5

Table S1. The molar ratios of each component in iLNP formulations tested in this study.

Name	Molar Percentage				LNP: siRNA (w/w)
	Ionizable lipid	DSPC	Cholesterol	DMG-PEG2000	
1	51.0%	10.2%	38.2%	0.6%	15:1
2	43.0%	12.9%	43.0%	1.1%	15:1
3	36.9%	14.7%	46.1%	2.3%	15:1
4	32.0%	16.0%	48.0%	4.0%	15:1
5	48.2%	8.2%	41.0%	2.6%	15:1
6	50.0%	12.8%	31.9%	5.3%	15:1
7	38.1%	13.0%	48.6%	0.4%	15:1
8	39.8%	16.9%	42.4%	0.8%	15:1
9	46.2%	6.8%	42.7%	4.3%	15:1
10	42.0%	9.3%	46.7%	1.9%	15:1
11	53.5%	15.8%	29.7%	1.0%	15:1
12	47.2%	17.5%	34.9%	0.4%	15:1
13	46.9%	6.2%	46.2%	0.8%	15:1
14	49.4%	9.7%	40.5%	0.4%	15:1
15	50.0%	13.1%	32.8%	4.1%	15:1
16	53.7%	17.6%	26.4%	2.2%	15:1

Table S2. The molar percentages of each component in iLNP formulations and the mass ratios between the iLNP and siRNA. The mass of iLNP means the sum of the masses of the four components.

Name	Mass Percentage			
	Ionizable lipid	DSPC	Cholesterol	DMG-PEG2000
A1-B3-7-1	74.6%	8.4%	15.4%	1.7%
A1-B3-7-2	67.2%	11.3%	18.5%	3.0%
A1-B3-7-3	59.5%	13.4%	20.4%	6.7%
A1-B3-7-4	52.1%	14.6%	21.5%	11.7%
A1-B3-7-5	**70.2%**	**6.7%**	**16.4%**	**6.7%**
A1-B3-7-6	66.2%	9.5%	11.6%	12.7%
A1-B3-7-7	64.1%	12.2%	22.5%	1.2%
A1-B3-7-8	63.7%	15.2%	18.6%	2.4%
A1-B3-7-9	66.5%	5.5%	16.9%	11.1%
A1-B3-7-10	66.1%	8.2%	20.2%	5.5%
A1-B3-7-11	74.0%	12.3%	11.3%	2.5%
A1-B3-7-12	70.0%	14.6%	14.2%	1.2%
A1-B3-7-13	72.8%	5.4%	19.7%	2.2%
A1-B3-7-14	74.1%	8.2%	16.7%	1.1%
A1-B3-7-15	67.8%	10.0%	12.2%	10.0%
A1-B3-7-16	71.8%	13.2%	9.7%	5.3%

Table S3. The mass percentage of the components of LNPs based on A1-B3-7.

Name	Mass Percentage			
	Ionizable lipid	DSPC	Cholesterol	DMG-PEG2000
A1-D1-5-1	66.8%	10.9%	20.0%	2.2%
A1-D1-5-2	58.5%	14.3%	23.4%	3.8%
A1-D1-5-3	50.2%	16.4%	25.1%	8.2%
A1-D1-5-4	42.8%	17.5%	25.7%	14.0%
A1-D1-5-5	61.8%	8.6%	21.0%	8.6%
A1-D1-5-6	57.4%	12.0%	14.6%	16.0%
A1-D1-5-7	55.0%	15.3%	28.1%	1.5%
A1-D1-5-8	54.7%	19.0%	23.3%	3.1%
A1-D1-5-9	57.7%	7.0%	21.3%	14.0%
A1-D1-5-10	57.2%	10.4%	25.4%	7.0%
A1-D1-5-11	66.1%	16.0%	14.7%	3.2%
A1-D1-5-12	61.6%	18.6%	18.2%	1.5%
A1-D1-5-13	**64.8%**	**6.9%**	**25.5%**	**2.8%**
A1-D1-5-14	66.2%	10.6%	21.7%	1.4%
A1-D1-5-15	59.1%	12.7%	15.5%	12.7%
A1-D1-5-16	63.6%	17.0%	12.5%	6.8%

Table S4. The mass percentage of the components of LNPs based on A1-D1-5.

Name	Mass Percentage			
	Ionizable lipid	DSPC	Cholesterol	DMG-PEG2000
A2-C1-8-1	72.7%	9.0%	16.5%	1.8%
A2-C1-8-2	65.0%	12.1%	19.7%	3.2%
A2-C1-8-3	57.1%	14.1%	21.6%	7.1%
A2-C1-8-4	49.7%	15.4%	22.6%	12.4%
A2-C1-8-5	68.1%	7.2%	17.6%	7.2%
A2-C1-8-6	64.0%	10.1%	12.4%	13.5%
A2-C1-8-7	61.8%	13.0%	23.9%	1.3%
A2-C1-8-8	61.4%	16.2%	19.8%	2.6%
A2-C1-8-9	64.2%	5.9%	18.0%	11.8%
A2-C1-8-10	63.8%	8.8%	21.5%	5.9%
A2-C1-8-11	72.0%	13.2%	12.1%	2.7%
A2-C1-8-12	67.9%	15.6%	15.2%	1.3%
A2-C1-8-13	70.8%	5.8%	21.1%	2.3%
A2-C1-8-14	**72.1%**	**8.8%**	**17.9%**	**1.2%**
A2-C1-8-15	65.6%	10.7%	13.0%	10.7%
A2-C1-8-16	69.8%	14.2%	10.4%	5.7%

Table S5. The mass percentage of the components of LNPs based on A2-C1-8.

Name	Mass Percentage			
	Ionizable lipid	DSPC	Cholesterol	DMG-PEG2000
A3-C1-8/D1-7-1	77.7%	7.3%	13.5%	1.5%
A3-C1-8/D1-7-2	70.9%	10.0%	16.4%	2.7%
A3-C1-8/D1-7-3	63.6%	12.0%	18.4%	6.0%
A3-C1-8/D1-7-4	56.4%	13.3%	19.6%	10.7%
A3-C1-8/D1-7-5	73.6%	5.9%	14.5%	5.9%
A3-C1-8/D1-7-6	70.0%	8.4%	10.3%	11.3%
A3-C1-8/D1-7-7	67.9%	10.9%	20.0%	1.1%
A3-C1-8/D1-7-8	67.6%	13.6%	16.6%	2.2%
A3-C1-8/D1-7-9	70.2%	4.9%	15.0%	9.9%
A3-C1-8/D1-7-10	69.8%	7.3%	17.9%	4.9%
A3-C1-8/D1-7-11	77.1%	10.8%	9.9%	2.2%
A3-C1-8/D1-7-12	73.5%	12.9%	12.6%	1.0%
A3-C1-8/D1-7-13	76.1%	4.7%	17.3%	1.9%
A3-C1-8/D1-7-14	77.2%	7.2%	14.6%	1.0%
A3-C1-8/D1-7-15	**71.4%**	**8.9%**	**10.8%**	**8.9%**
A3-C1-8/D1-7-16	75.1%	11.6%	8.5%	4.7%

Table S6. The mass percentage of the components of LNPs based on A3-C1-8/D1-7.

Name	Size	PDI	E.E.	L.E.
A1-B3-7-1	171.27±13.71	0.125±0.080	93%	62%
A1-B3-7-2	124.63±2.54	0.134±0.010	95%	69%
A1-B3-7-3	160.50±4.09	0.170±0.039	94%	70%
A1-B3-7-4	136.47±2.35	0.190±0.040	94%	63%
A1-B3-7-5	**186.40±3.15**	**0.191±0.022**	**91%**	**62%**
A1-B3-7-6	131.47±1.82	0.151±0.014	91%	67%
A1-B3-7-7	184.40±6.59	0.141±0.013	91%	58%
A1-B3-7-8	150.87±2.51	0.217±0.025	91%	62%
A1-B3-7-9	131.00±1.14	0.130±0.027	89%	64%
A1-B3-7-10	182.03±3.65	0.201±0.017	92%	65%
A1-B3-7-11	187.37±4.61	0.130±0.066	90%	63%
A1-B3-7-12	198.17±2.31	0.188±0.025	90%	53%
A1-B3-7-13	160.47±4.76	0.169±0.029	85%	62%
A1-B3-7-14	164.37±7.54	0.178±0.034	90%	60%
A1-B3-7-15	157.83±2.15	0.152±0.028	89%	69%
A1-B3-7-16	166.90±4.81	0.161±0.024	90%	54%

Table S7. Physiochemical characterizations of siRNA-loaded iLNPs based on A1-B3-7, including the particle size, polydispersity index (PDI), encapsulation efficiency (E.E.) and loading efficiency (L.E.).

Name	Size	PDI	E.E.	L.E.
A1-D1-5-1	129.90±0.71	0.124±0.028	91%	58%
A1-D1-5-2	106.35±0.49	0.133±0.008	92%	58%
A1-D1-5-3	97.16±0.37	0.130±0.007	91%	52%
A1-D1-5-4	92.25±0.32	0.143±0.004	89%	45%
A1-D1-5-5	137.10±0.99	0.278±0.011	90%	46%
A1-D1-5-6	120.50±0.42	0.136±0.005	86%	53%
A1-D1-5-7	128.30±0.14	0.081±0.016	90%	43%
A1-D1-5-8	187.30±1.41	0.133±0.001	87%	33%
A1-D1-5-9	104.10±0.99	0.127±0.010	92%	65%
A1-D1-5-10	106.35±0.92	0.132±0.004	91%	59%
A1-D1-5-11	139.90±1.41	0.135±0.008	91%	66%
A1-D1-5-12	202.55±1.91	0.026±0.022	89%	52%
A1-D1-5-13	**163.07±2.87**	**0.125±0.022**	**94%**	**77%**
A1-D1-5-14	138.30±0.28	0.061±0.005	94%	84%
A1-D1-5-15	113.55±0.35	0.145±0.008	91%	66%
A1-D1-5-16	123.65±1.06	0.171±0.018	92%	64%

Table S8. Physiochemical characterizations of siRNA-loaded iLNPs based on A1-D1-5, including the particle size, polydispersity index (PDI), encapsulation efficiency (E.E.) and loading efficiency (L.E.).

Name	Size	PDI	E.E.	L.E.
A2-C1-8-1	185.33±3.32	0.166±0.054	92%	85%
A2-C1-8-2	203.83±4.30	0.198±0.023	89%	61%
A2-C1-8-3	158.83±1.99	0.126±0.010	89%	90%
A2-C1-8-4	99.98±2.12	0.149±0.018	89%	78%
A2-C1-8-5	165.53±2.20	0.157±0.020	94%	93%
A2-C1-8-6	110.77±0.40	0.196±0.006	91%	91%
A2-C1-8-7	187.60±2.52	0.204±0.025	90%	85%
A2-C1-8-8	163.97±1.01	0.185±0.005	85%	91%
A2-C1-8-9	112.27±1.29	0.167±0.016	86%	59%
A2-C1-8-10	136.57±2.50	0.157±0.003	88%	43%
A2-C1-8-11	136.67±1.36	0.133±0.070	85%	89%
A2-C1-8-12	164.07±2.13	0.133±0.052	91%	57%
A2-C1-8-13	172.67±1.62	0.167±0.019	87%	65%
A2-C1-8-14	**177.67±6.50**	**0.136±0.096**	**84%**	**93%**
A2-C1-8-15	96.71±0.23	0.160±0.021	90%	79%
A2-C1-8-16	177.40±5.98	0.167±0.009	92%	86%

Table S9. Physiochemical characterizations of siRNA-loaded iLNPs based on A2-C1-8, including the particle size, polydispersity index (PDI), encapsulation efficiency (E.E.) and loading efficiency (L.E.).

Name	Size	PDI	E.E.	L.E.
A3-C1-8/D1-7-1	184.23±2.55	0.104±0.026	90%	17%
A3-C1-8/D1-7-2	175.50±2.46	0.098±0.017	87%	28%
A3-C1-8/D1-7-3	171.87±1.32	0.107±0.034	84%	29%
A3-C1-8/D1-7-4	152.97±1.01	0.127±0.018	90%	43%
A3-C1-8/D1-7-5	159.37±0.70	0.101±0.017	83%	37%
A3-C1-8/D1-7-6	176.27±1.42	0.117±0.027	85%	29%
A3-C1-8/D1-7-7	159.20±2.54	0.091±0.033	95%	32%
A3-C1-8/D1-7-8	165.07±3.02	0.105±0.037	92%	31%
A3-C1-8/D1-7-9	139.37±1.51	0.134±0.054	85%	45%
A3-C1-8/D1-7-10	153.20±0.20	0.129±0.035	82%	37%
A3-C1-8/D1-7-11	151.70±1.47	0.138±0.008	96%	24%
A3-C1-8/D1-7-12	159.30±5.99	0.213±0.025	95%	24%
A3-C1-8/D1-7-13	162.97±2.17	0.115±0.020	94%	26%
A3-C1-8/D1-7-14	179.70±2.00	0.125±0.032	95%	19%
A3-C1-8/D1-7-15	**133.23±1.48**	**0.127±0.037**	**86%**	**42%**
A3-C1-8/D1-7-16	164.27±1.12	0.150±0.017	84%	43%

Table S10. Physiochemical characterizations of siRNA-loaded iLNPs based on A3-C1-8/D1-7, including the particle size, polydispersity index (PDI), encapsulation efficiency (E.E.) and loading efficiency (L.E.).

Name (siRNA)	Sequence (5′-3′)
siApoB	(s): GmsUmsCmAmUfCmAfCfAfCmUmGmAmAmUmAmCmCmAm (as): msGfsGmUmAmUfUmCfAfGmUmGmUmGfAmUfGmAmCmsAmsCm
siANGPTL3	(s): msCmsAmUmAmUmUfUmGfAfUfCmAmGmUmCmUmUmUmUmUm (as): msAfsAmAmAmGfAmCmUmGmAmUmCmAfAmAfUmAmUmGmUmsUmsGm
siApoC3	Refer to the patent: WO2019105419 (A1)
siSCD1	Refer to the literature: Theranostics 2016;6(10):1528-41
siFL	(s): CmCmCmUmAUUCUmCCmUUmCmUmUmCGCdTsdT (as): GfCmGfAAGAAfGGAGAAfUAGGGdTsdT
siNC	(s): UmUCmUCmCGAACGUGmUCmAmCGUdTdT (as): ACmGUfGACfACGUUCfGGAGAAdTdT

Table S11. Sequence information of siRNAs used in this work. (s), sense strand, (as), antisense strand. Chemical modifications: m, 2'-OMe, f, 2'-F, s, phosphorothioate. siFL, siRNA against firefly luciferase gene.

Name (primer)	Sequence (5′-3′)
h-GAPDH	(f): AGAAGGCTGGGGCTCATTTG (r): AGGGGCCATCCACAGTCTTC
m-GAPDH	(f): AACTTTGGCATTGTGGAAGGGCTC (r): TGGAAGAGTGGGAGTTGCTGTTGA
h-ApoB	(f): GCCAGTCCTTCATGTCCCTA (r): TGAAGAAAGGAGATGAGCAACAA
m-ApoB	(f): TTCCAGCCATGGGCAACTTTACCT (r): TACTGCAGGGCGTCAGTGACAAAT
m-ANGPTL3	(f): GAGGAGCAGCTAACCAACTTAAT (r): TCTGCATGTGCTGTTGACTTAAT
h-ApoC3	(f): TGGGTCCTGCAATCTCCA (r): TTTTACTCATAGCAGCTTCTTGT
m-SCD1	(f): TGGTGAACAGTGCCGCGCAT (r): ACTCAGAAGCCCAAAGCTCAGCTAC

Table S12. Sequence information of primer sets used in this work. (f), forward primer; (r), reverse primer.