OMTN, Volume *19*

Supplemental Information

Efficient Delivery of Antisense Oligonucleotides Using Bioreducible Lipid Nanoparticles *In Vitro* and *In Vivo*

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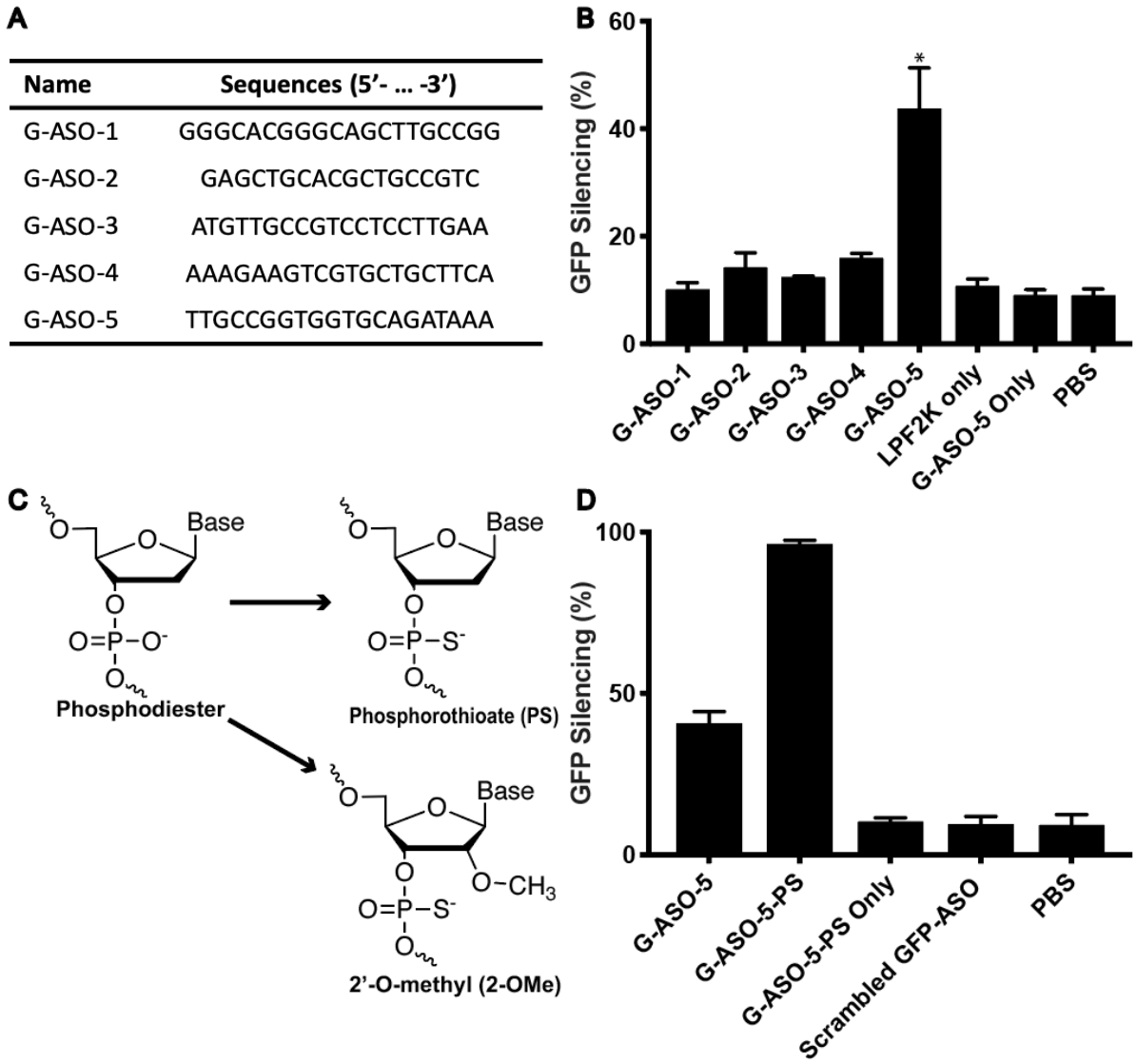

Figure S1 Sequences and chemistry design of ASOs. (A) Design of five regular 20-nucleotides lengths of ASOs (G-ASO-1 to G-ASO-5). (B) Screening of the ASO sequences with the highest delivery efficiency. All the GFP-ASO were delivered to GFP-HEK cell lines with LPF 2000. The 5th ASO fragment exhibited 43.7% of the GFP silencing efficacy, outperforming than that of other ASO sequences, *$p < 0.01$ vs. all different samples. (C) Scheme of further modifications of ASO linkages and ribose. We chose the 5th ASO sequence to further enhance their resistance towards endonucleases and stability by optimizing linkages to phosphonothioate bonds and modifying ribose of first and last five nucleic acids with 2'-O-methyl (2-OMe). (D) Comparison of the 5th ASO with phosphodiester linkages and ASOs containing PS bonds and 2-OMe. The modified 5th ASO showed 55.6% of the increase in GFP mRNA repression, demonstrating that the chemical modification played a crucial role in improving the capacity and stability in ASOs. The chemically modified scrambled-ASO delivered by LPF 2000 showed no effect in GFP silencing, indicating ASO sequence-specific gene silence, *$p < 0.01$ vs. all other samples. The GFP intensity was then analyzed by flow cytometry after 16 h of post-delivery. Data are presented as mean ± SD ($n \geq 3$).

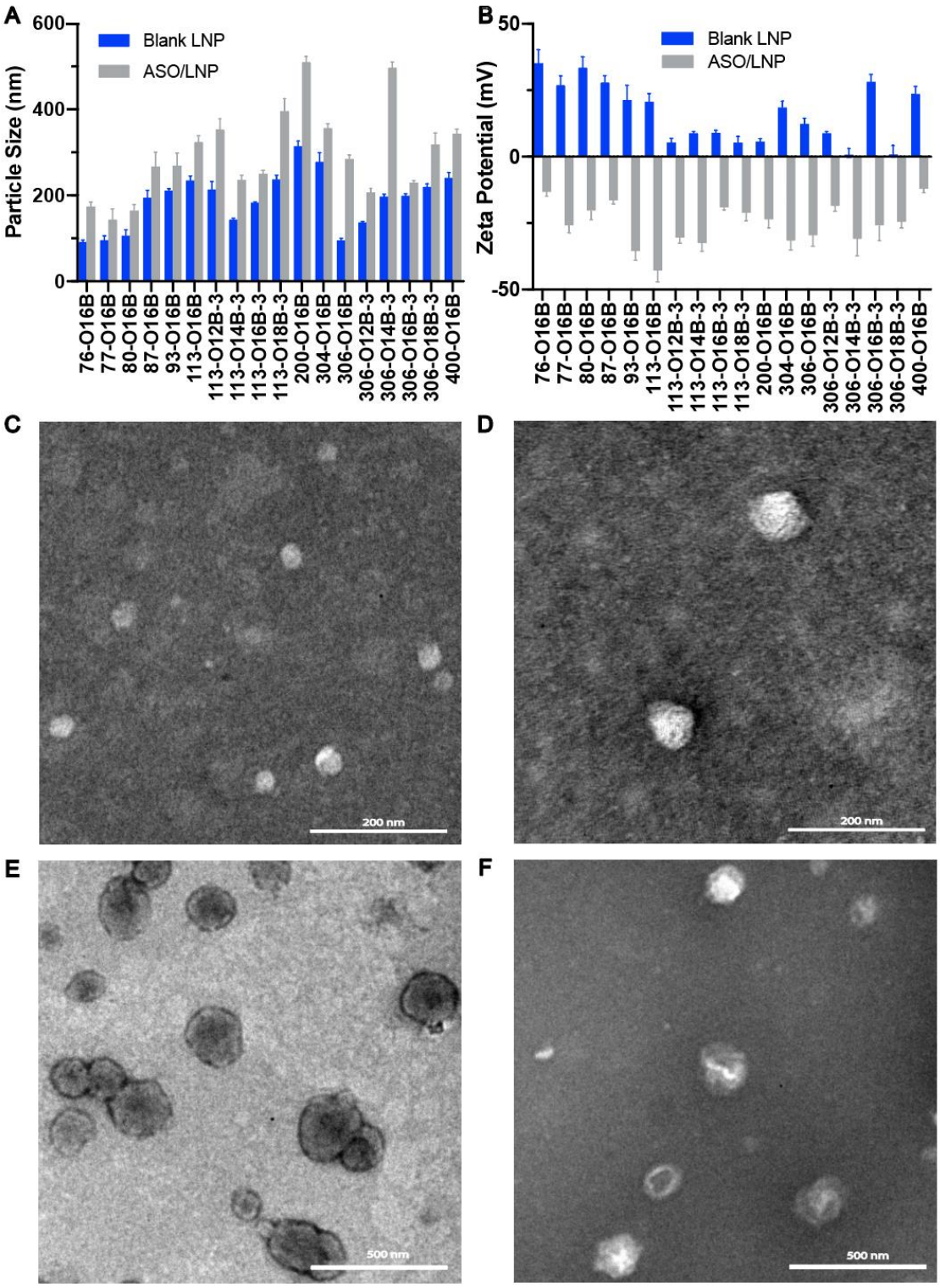

Figure S2 Characterization of bioreducible lipid nanoparticles. (A) Hydrodynamic size distributions of blank or ASO loaded nanoparticles with no formulation, determined by dynamic light scattering (DLS). The increased sizes indicated the successful load of ASO. (B) Zeta-potential profile of blank or ASO loaded nanoparticles with no formulation. The decrease of the zeta-

potential indicated the successful encapsulation of ASO. (C) The TEM image of blank 306-O12B-3 LNPs with no formulation showing uniform spheres in diameters around 100 nm. (D) The TEM image of ASOs/306-O12B-3 LNPs complexes (with no formulation), showing uniform spheres in diameters of 200 nm. (E) Blank 306-O12B-3 LNPs, formulated with cholesterol, DSPE, and DSPE-PEG2000 at the weight ratio of 16/4/1/1. The results showed uniform spheres in diameters around 300 nm. (F) The TEM image of ASOs/306-O12B-3 LNPs complexes (formulated with cholesterol, DSPE, and DSPE-PEG2000 at the weight ratio of 16/4/1/1), showing no significant change in complexes sizes.

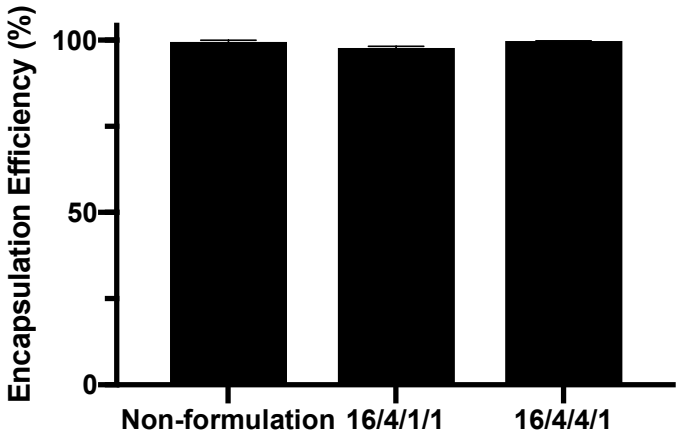

Figure S3 The ASO encapsulation efficiencies of different lipid formulation: Non-formulated 306-O12B-3 LNPs for *in vitro* screening, 306-O12B-3 LNPs formulated with cholesterol, DSPE, and DSPE-PEG2000 at the weight ratio of 16/4/1/1, and 306-O12B-3 LNPs formulated with cholesterol, DSPE, and DSPE-PEG2000 at the weight ratio of 16/4/4/1.

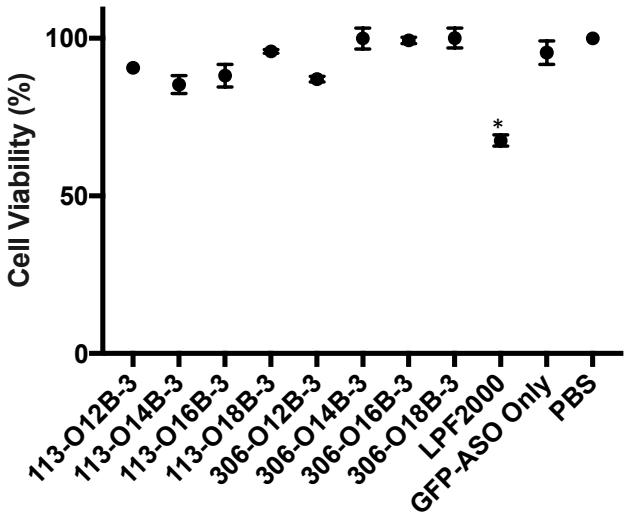

Figure S4 Cytotoxicity analysis of all our three tails lipids comparing to that of LPF 2000. All our three tails lipids resulted in low cytotoxicity which was demonstrated by the cell viability after GFP-ASO/LNPs complexes treatments all maintained above 85.3%, the same cell proliferation as control groups (cells treated by PBS only). The GFP-ASO with no LNPs encapsulated were used to treated GFP-HEK cells to exclude the possibility that ASO caused the toxic effects. Meanwhile, the LPF2000 exhibited 67.6% of the cell viability which was significantly lower than that of our lipids, indicating that our lipids were safer than the commercial transfection reagent LPF 2000 at the same doses, *p< 0.01 vs. all other samples.

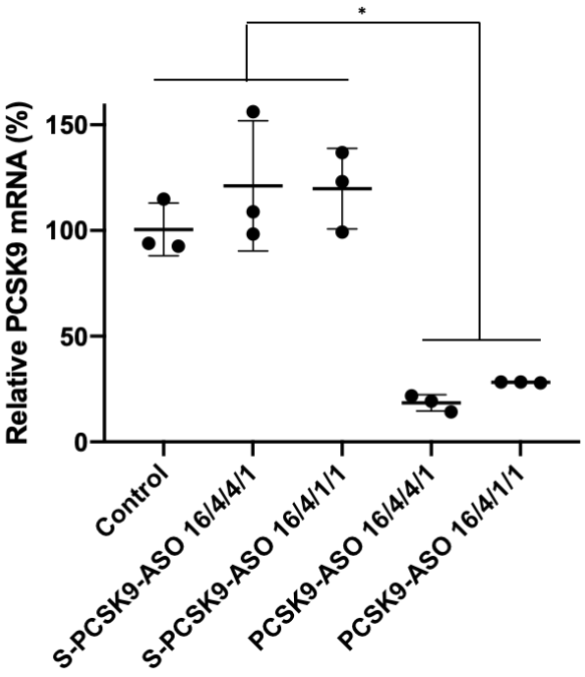

Figure S5 Comparison of the PCSK9 mRNA silencing effect by scrambled PCSK9-ASO and functional PCSK9-ASO *in vivo*. Each dot represents the PCSK9 mRNA silencing level in each mouse received 1.5 mg kg^{-1} of PCSK9-ASO. Both ASOs were delivered by the best-performing lipid, 306-O12B-3, at two different formulations (cationic lipid/cholesterol/DOPE/DSPE-PEG2000 at the ratio of 16/4/4/1 or 16/4/1/1 wt/wt). No matter in which formulation, no PCSK9 silencing effect were observed, demonstrating the specific targeting of functional PCSK9-ASO *in vivo*, *p< 0.01.

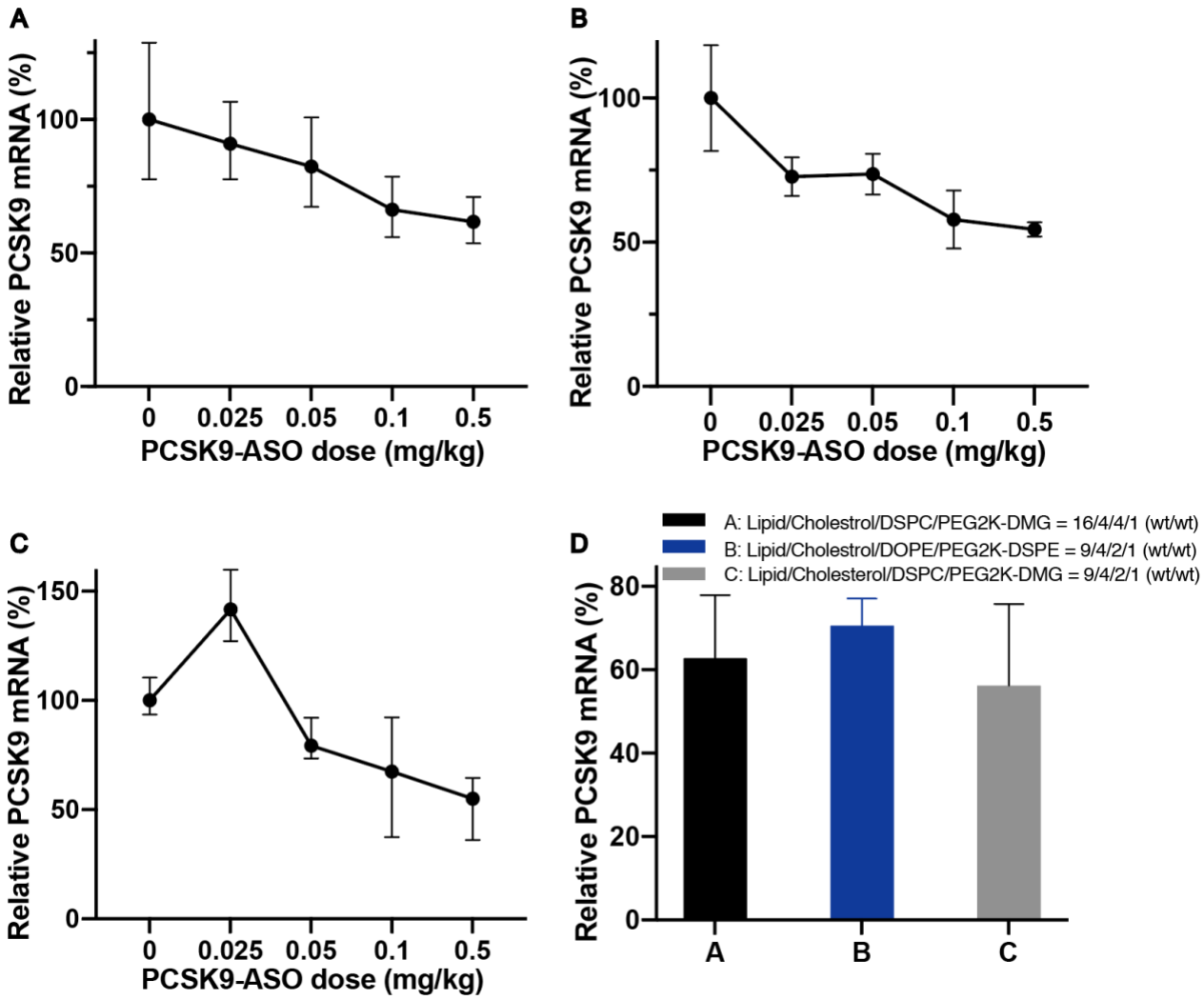

Figure S6 Different formulations for lipid 306-O12B-3 in PCSK9-ASO delivery *in vivo*. To further improve the PCSK9-ASO delivery efficiency, the lipid composition and ratios were modified. (A) Based on the original lipid formulation (cationic lipid/cholesterol/DSPE/DSPE-PEG2000 at the weight ratio of 16/4/1/1), the weight ratio of cholesterol was increased from 4 to 8 without changing any ratios or components of other helper lipids. Animals received LNPs/ASO complexes injection via tail vein at PCSK9-ASO doses of 0.025, 0.05, 0.1 or 0.5 mg kg^{-1}. (B) We improved the weight ratio of cholesterol from 4 to 12. (C) The weight ratio of lipid/cholesterol/DOPE/DSPE-PEG2000 were all doubled with the injective formulation as 8/4/1/1. (D) Mice received IV injections at ASO dose of 0.0343 mg kg^{-1} which was the ED_{50} of optimal formulation 16/4/4/1. Results showed that none of the lipid formulations demonstrated ED_{50} lower than 50%. The control group was injected with saline. Mice were sacrificed after 72 h of post-injection. PCSK9 mRNA levels relative to β-actin mRNA levels were determined in liver samples. Data points are expressed as a percentage of saline control animals and represent group mean ± SD (n=3).

Table S1. Radiant efficiency of the far-red fluorescent intensity in different organs. Mice were treated with ASO-Alexa750/LNPs complexes.

Radiant Efficiency* / Groups	Brain	Heart	Lung	Liver	Spleen	Kidney
113-O14-3	0.7278	0.5150	15.33	49.51	1.863	2.13
113-O16-3	0.7619	0.5216	13.38	90.62	5.985	5.57
306-O12-3	0.7367	0.6031	6.749	136.9	8.531	4.876
Saline	0.4686	0.1951	0.5788	1.574	0.3903	0.5301

The asterisk (*) presents units of radiant efficiency x10^9 (p/sec/cm^2/sr)/($\mu W/cm^2$)