Supporting information

Elucidation of the physicochemical properties and potency of siRNA-loaded small-sized lipid nanoparticles for siRNA delivery

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Table S1. siRNA sequences used in this study.

siRNA	sense strand (5' → 3')	antisense strand (5' → 3')
siGL4	CCGUCGUCUUCGUGAGCAATT	UUGCUCACGAAUACGACGGTT
siPLK1	AGAuCACCCuCCUUAAAuAUU	UAUUUAAGGAGGGUGAuCUUU
siFVII	CGA**uc**A**ucuc**AAG**ucuu**ACT*T	G**u**AAGA**cuu**GAGA**u**GA**ucc**T*T
Cy5-siGFP	AcAuGAAGcAGcACGACuUT*T	AAGUCGUGCUGCUUCAUGUT*T-Cy5
Cy3-siGL4	CCGUCGUAUUCGUGAGCAATT	Cy3-UUGCUCACGAAUACGACGGTT

2'-OMe modified nucleotides are depicted in lower case letters, 2'-fluoro modified nucleotides are in bold lower case letters, and phosphorothioate linkages are represented by asterisks.

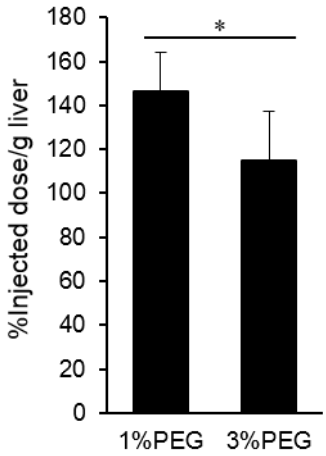

Figure S1. Quantification of siRNAs in liver tissues. ICR mice were intravenously administered with Cy5-siGFP formulated in the LNPs at a dose of 0.5 mg/kg. Thirty minutes after administration, liver tissues were collected and homogenized in a 1%SDS solution. Fluorescence derived from Cy5 in liver homogenates was measured using a spectrofluorometer (Tecan Infinite 200, Tecan Austria GmbH, Austria). The amount of siRNA is indicated as the percentage of the injected dose per unit gram liver tissue. n=5-6. Data are represented as the mean±SD. *P<0.05.

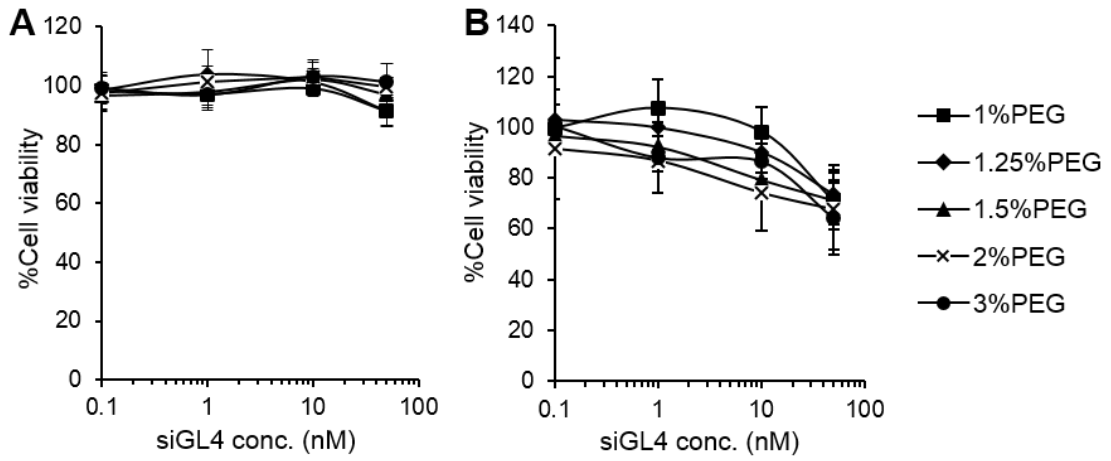

Figure S2. *In vitro* cytotoxicity of the LNPs. HeLa-dluc cells were transfected with siGL4 containing LNPs with different amount of PEG-DMG in the presence of serum (A) or in the absence of serum (B). Twenty-four hours after transfection, the cells were lysed, and Renilla luciferase activity was measured as an indicator of the number of cells. n=3-4. Data are represented as the mean±SD.

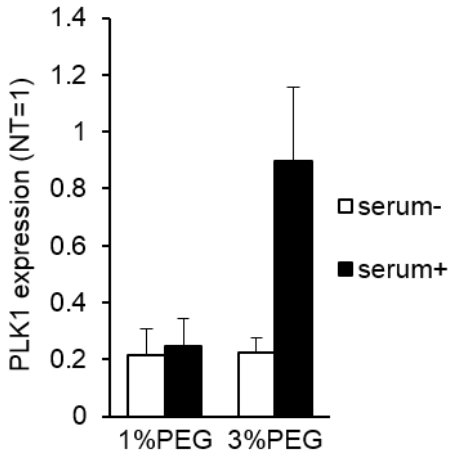

Figure S3. *In vitro* gene silencing activity of the LNPs. Huh7 cells, human hepatocellular carcinoma, were transfected with siRNA against polo-like kinase 1 (siPLK1) containing 1%PEG-LNPs and 3%PEG-LNPs in the presence or absence of serum. Twenty-four hours after transfection, PLK1 mRNA expression was measured by quantitative RT-PCR. n=3. Data are represented as the mean±SD.

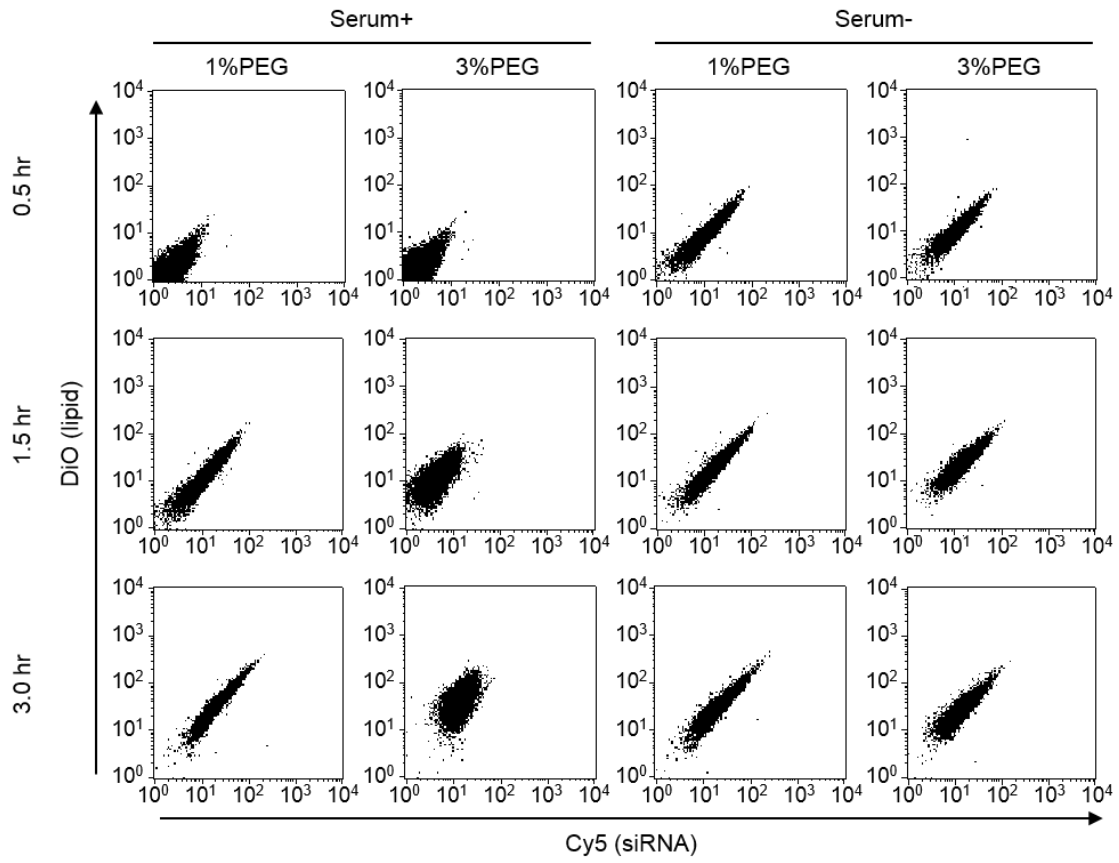

Figure S4. Dot-plots of Cy5 and DiO. HeLa-dluc cells were transfected with dual-labeled LNPs (Cy5 for siRNAs, DiO for lipids) for the indicated times. Cellular uptake was measured by flow cytometry.

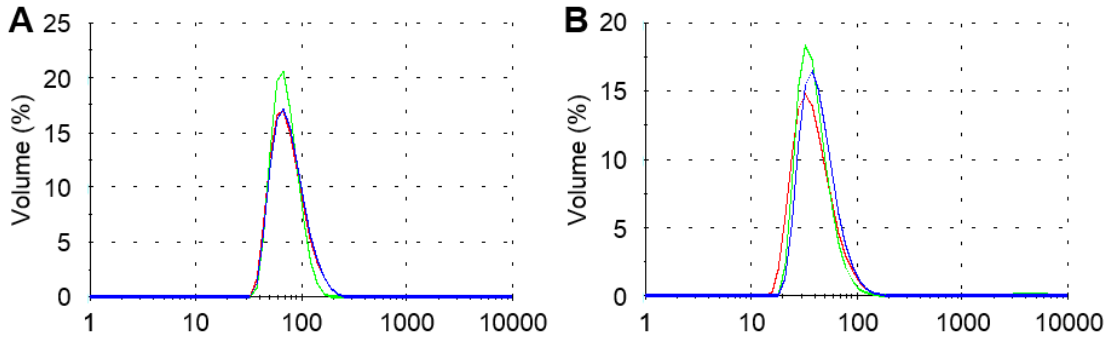

Figure S5. Size distribution of the 1%PEG-LNPs (A) and 3%PEG-LNPs (B) before and after mixing with serum. The size distribution of the LNPs before mixing, 5 minutes after mixing, and 30 minutes after mixing are represented as red, green and blue, respectively.

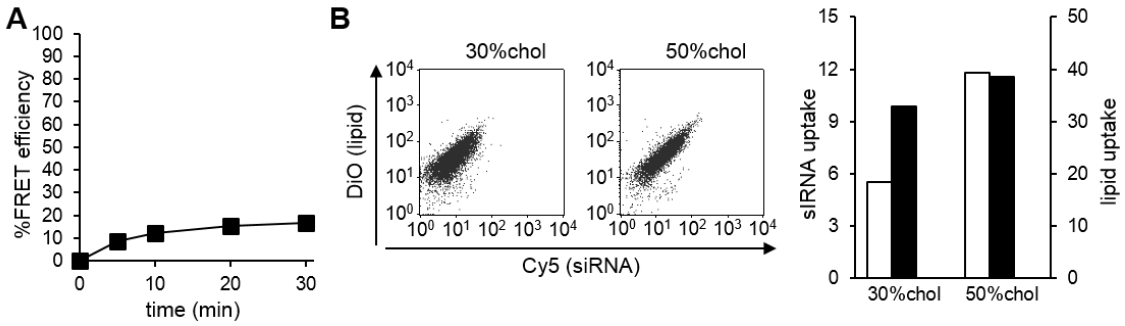

Figure S6. Evaluation of the stability of cholesterol-rich, small-sized LNPs in the presence of serum. (A) Leakage of siRNAs was detected by cancellation of siRNA FRET. n=2. (B) Cellular uptake of siRNAs and lipids. HepG2 cells, human hepatocellular carcinoma, were transfected with dual-labeled 3%PEG-LNPs (Cy5 for siRNAs, DiO for lipids) for 3 hours, and then analyzed by flow cytometry. n=1.

Data are represented as the mean.