Supplementary Information

PEG-OligoRNA hybridization of mRNA for developing sterically stable lipid nanoparticles toward *in vivo* administration

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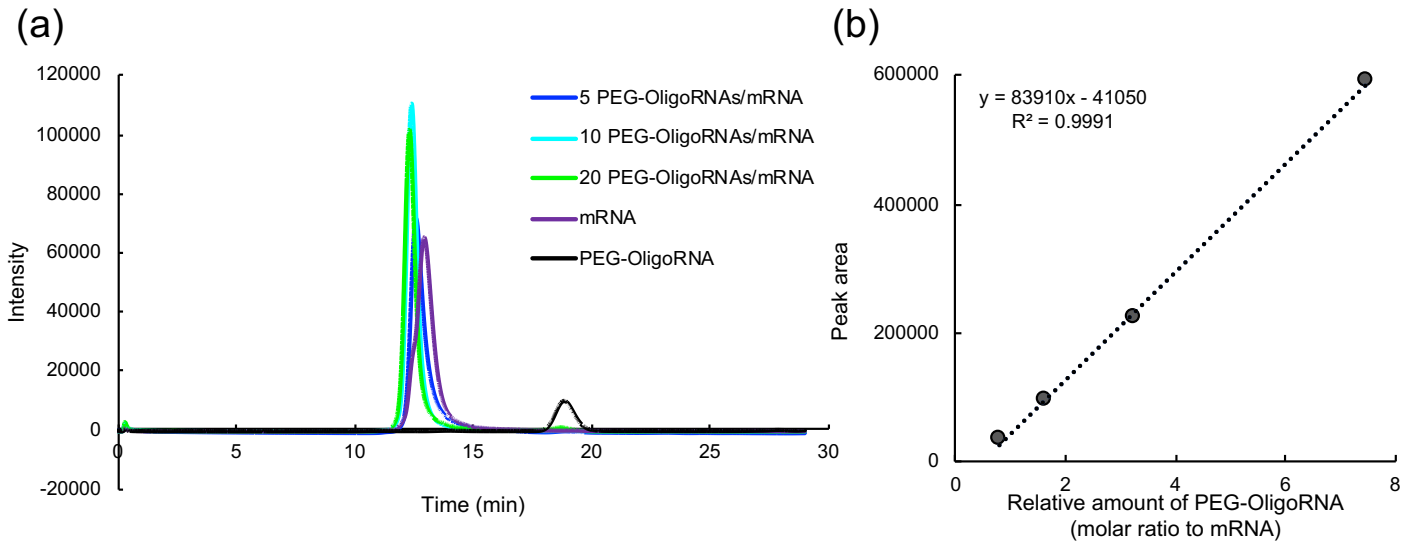

Figure S1. Gel permeation chromatography (GPC) for evaluation of hybridization efficiency. GPC was performed using JASCO UV-4075 (JASCO, Tokyo, Japan) equipped with Sephadex 200 Increase 10/300 GL column (GE Healthcare UK Ltd., Buckinghamshire, UK) in 10 mM PBS at flow rate of 0.75 ml/min. (a) GPC chart of mRNA hybridized with 5, 10 or 20 PEG-OligoRNAs, free mRNA and free PEG-OligoRNA. The peaks observed in 12 – 15 min are derived from mRNA or PEG-OligoRNAs/mRNA, and those in 18 – 20 min are from free PEG-OligoRNA. (b) Peak area from PEG-OligoRNA with different concentration. The amount of free PEG-OligoRNA in the solution of PEG-OligoRNAs/mRNA was calculated based on peak area from free PEG-OligoRNA in (a) and standard curve prepared in (b).

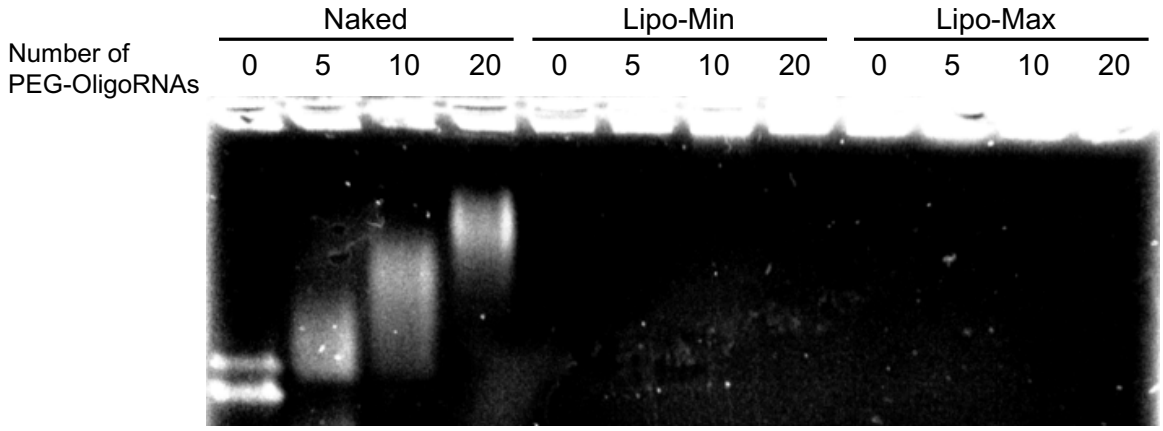

Figure S2. Gel electrophoresis of lipofectamine LTX/mRNA LNPs. Two LNP formulations were prepared by mixing mRNA with minimal or maximal volume of lipofectamine LTX solution that the manufactures suggest to use (Lipo-Min and Lipo-Max, respectively). Unhybridized mRNA and mRNA hybridized with 5, 10, or 20 PEG-OligoRNAs were subjected to electrophoresis, in a naked form or in a form of Lipo-Min or Lipo-Max.

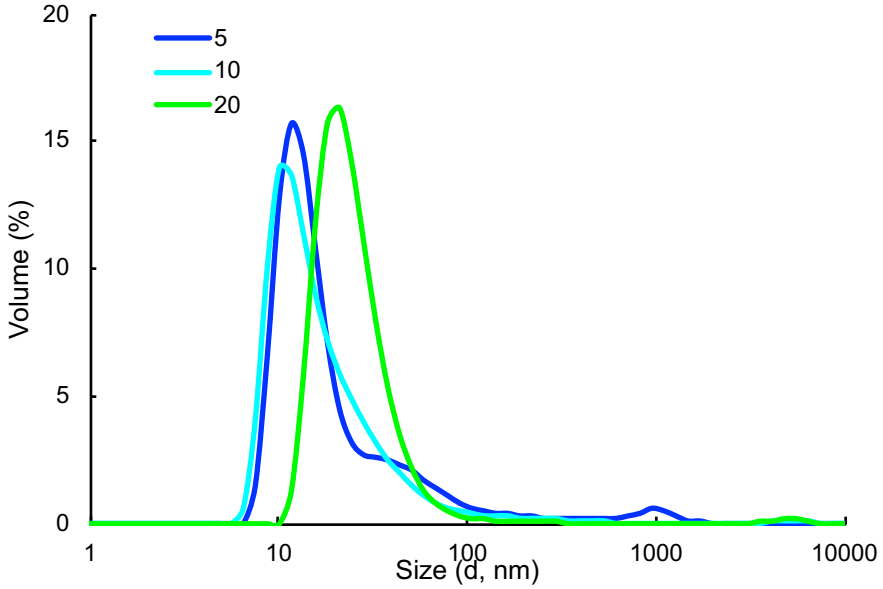

Figure S3. Dynamic light scattering (DLS) measurement of Lipofectamine LTX added with PEG-OligoA. PEG-OligoA was added to Lipofectamine LTX without the presence of mRNA. The concentration of PEG-OligoA and Lipofectamine LTX in this experiment was adjusted to the same as that of PEG-OligoRNAs and Lipofectamine LTX used to obtain **Figure 4d**. The numbers, 5, 10, and 20 in this figure, represent eq. of PEG-OligoA relative to mRNA concentration in the experiment of **Figure 4d**, respectively.

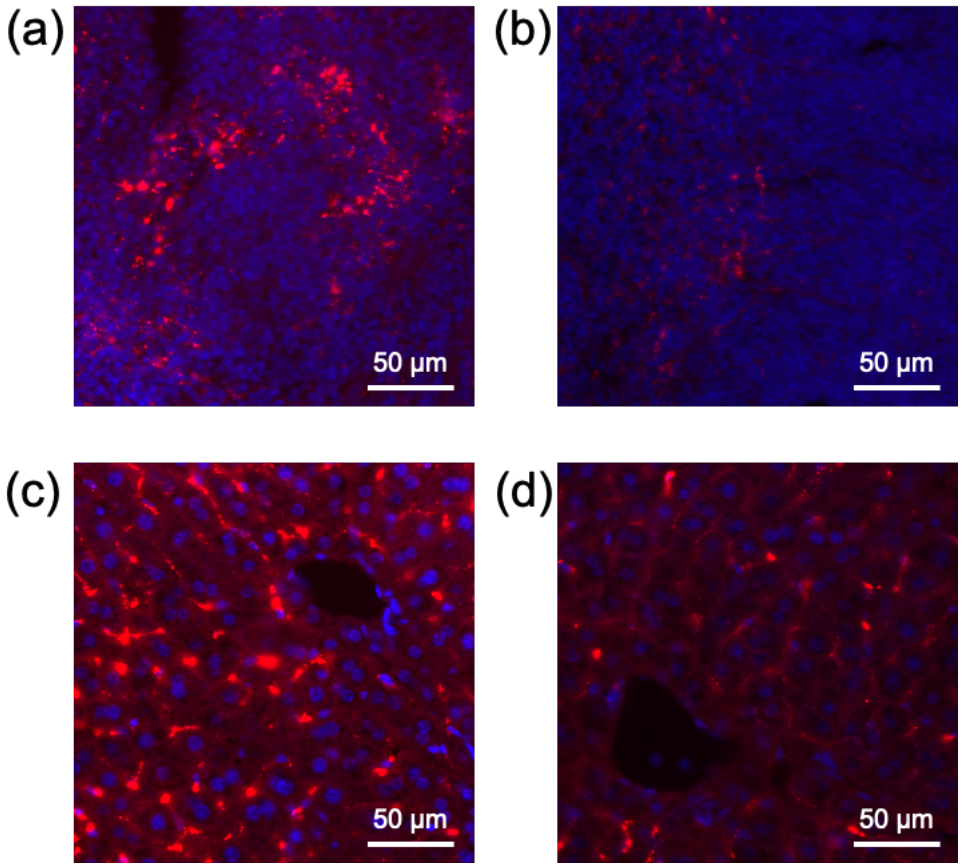

Figure S4. Distribution of Lipo-Max in the spleen and the liver. Lipo-Max prepared from Cy5-labeled mRNA (red) was intravenously injected to the mice. Five min after the injection, (**a**, **b**) the spleen and (**c**, **d**) the liver was excised for preparation of frozen tissue sections. Blue: nucleus (DAPI). (**a**, **c**) Lipo-Max loaded with non-PEGylated mRNA. (**b**, **d**) Lipo-Max loaded with 20 PEG-OligoRNAs/mRNA.

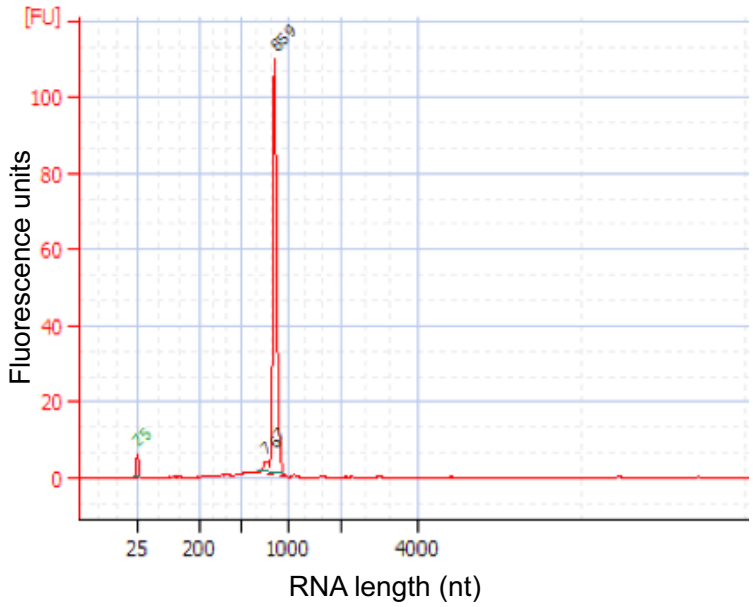

Figure S5. Capillary electrophoresis of *GLuc* mRNA. *GLuc* mRNA was subjected to the electrophoresis after mixed with 25 nt RNA marker.

1 GGGAGACCGGCCUCGAGCAGCUGAAGCUUGGUACCGAGCUCGGAUCCAGCCACCAUGG**GAGUCAAAGUUCUGUUU**GCCCUGAUCU**GCAUCGCUGUGGCCG**

1 ((....)).[[...((((.)).....))]]..[[[[[[[[[[[[[[..(((((.(((.....................(((((((.....((((.[

101 **A**GGCCAAGCCCACCGAG**AACAACGAAGACUUCAA**CAUCGUGGCCGUGG**CCAGCAACUUCGCGACC**ACGGAUCUCGAUGCUG**ACCGCGGGAAGUUGCCC**GG

101 [[]]][[..[[...[[[..........((((..((((((...((((((...([[[....]]..)))))))....)))))).........))))))....((

201 CAAGAAGCUGCCG**CUGGAGGUGCUCAAAGA**GAUGGAAGCCAAUG**CCCGGAAAGCUGGCUGC**ACCAGGGGCUGUC**UGAUCUGCCUGUCCCAC**AUCAAGUGC

201 (........)))...[[[.[[[.[[[[...[[.[[[......((.....))..

301 ACGCCCA**AGAUGAAGAAGUUCAUC**CCAGGACGCUGCCACACCUA**CGAAGGCGACAAAGAGU**CCGCACAGGGCGGCAUAGGCGAGGCGAUC**GUCGACAUUC**

301 ........((((((....))))))........[[[[[[.............................((.))]]]]...(((((()))))]]]]]]]]]]]]

401 **CUGAGAU**UCCUGGGUUCAAGGACU**UGGAGCCCAUGGAGCAG**UUCAUCGC**ACAGGUCGAUCUGUGUG**UGGACUGC**ACAACUGGCUGCCUCAA**AGGGCUUG**C**

401 [[[[[..[[[[[[[[(...)))))))]]]]]]]]]].(((((((((.(((((((((....)))))))))))))))))).....(((.(())...))]]]]]]

501 **CAACGUGCAGUGUUCU**GACCUGCU**CAAGAAGUGGCUGCCGC**AACGCUGUG**CGACCUUUGCCAGCAAG**AUCCAGGG**CCAGGUGGACAAGAUCA**AGGGGGCC

501)..[[[[[[[[((((..))..........................))]]]]]]]]..]]].........)))..)))))))))))))).....]].....((((

601 GGUGGUGACUAAGCGGCCGCU**CGAGCAUGCAUCUAGAG**GAUCCCCGG**GUACCGAGCUCGAAUUC**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

601 (............)))))..........................))]]]]))))))))).....................................

701 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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Table S1. Secondary structure in *GLuc* mRNA sequence. The structure was predicted using IPKnot software (http://rtips.dna.bio.keio.ac.jp/ipknot/). “( )” , “[ ]” showed base paring and “..” showed the bases without base pairing. Bold characters represent the sequence to which complementary PEG-OligoRNAs were designed.