SUPPLEMENTARY INFORMATION

Lipid-mRNA Nanoparticle Designed To Enhance Intracellular Delivery Mediated By Shock Waves

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Supplementary Information Index

Figure S1

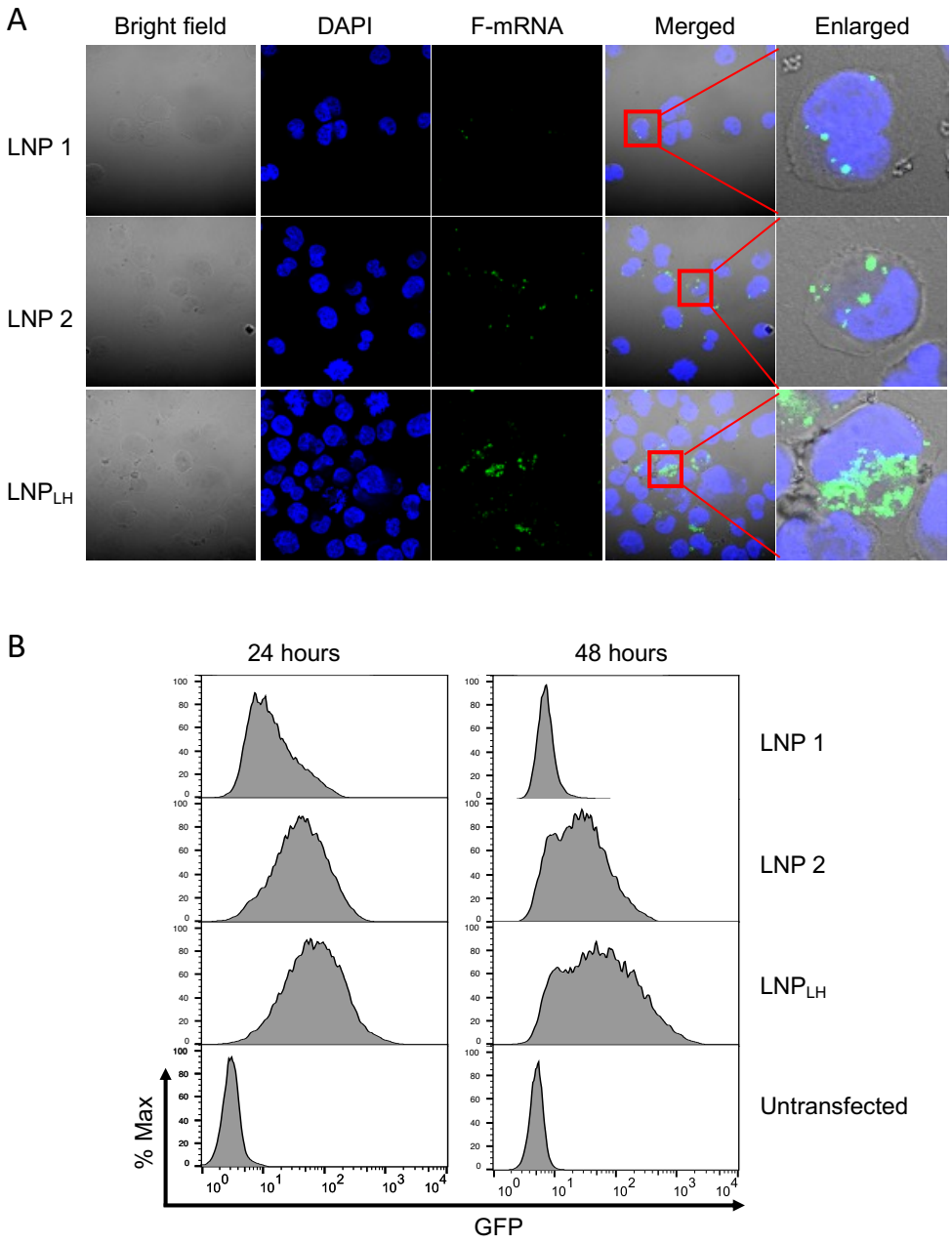

Figure S1. mRNA Delivery Using Different Formulations Of LNP.
A total of 600 ng of fluorescently labeled mRNA synthesised with fluorescein-UTP and ACAR cap were used to transfected 90,000 of HEK293T cells using LNP1, LNP2 or LNP$_{LH}$ at the mRNA:LNP ratio of 20:100 (w/w). The fluorescent signal from the transfected cells were analyzed 24 hours after transfection using confocal microscopy(A) or 24 and 48 hours after transfection using FACS (B).

Figure S2

Sample	H_2O	Lipoplex	Luciferase Control RNA	Recovery 1	Recovery 2	mRNA
Total mRNA	0	0.3 µg	2 µg	0.7 µg	2 µg	2 µg

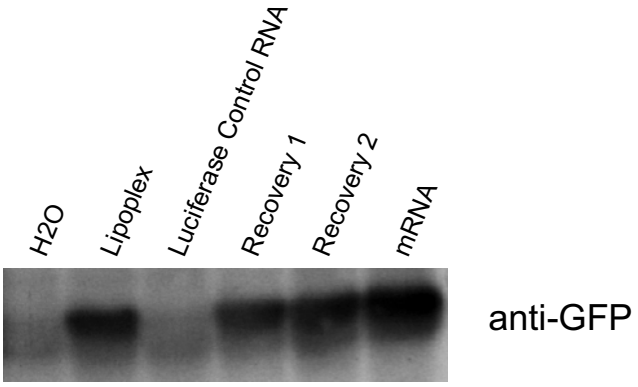

Figure S2. mRNA Is Intact In The LNP_{LH}/mRNA Lipoplex.
The integrity of mRNA in the LNP complex was analyzed by extracting mRNA from LNP_{LH}/mRNA lipoplex and testing in protein translation *in vitro* using a rabbit reticulocyte lysate system. A LNP_{LH}/mRNA lipoplex (lipoplex made at mRNA:LNP_{LH} ratio of 20:100 w/w) and the mRNA purified from the complex in duplicate (Recovery 1 and Recovery 2) using RNeasy Mini Kit (Qiagen 74104) were used as templates for protein translation in comparison to translation of starting mRNA and an mRNA-encoding luciferase. The amount of mRNA templates used for protein translation is indicated in the table. The translation reactions were fractionated by SDS-PAGE and western blotted with an anti-GFP antibody.

Figure S3

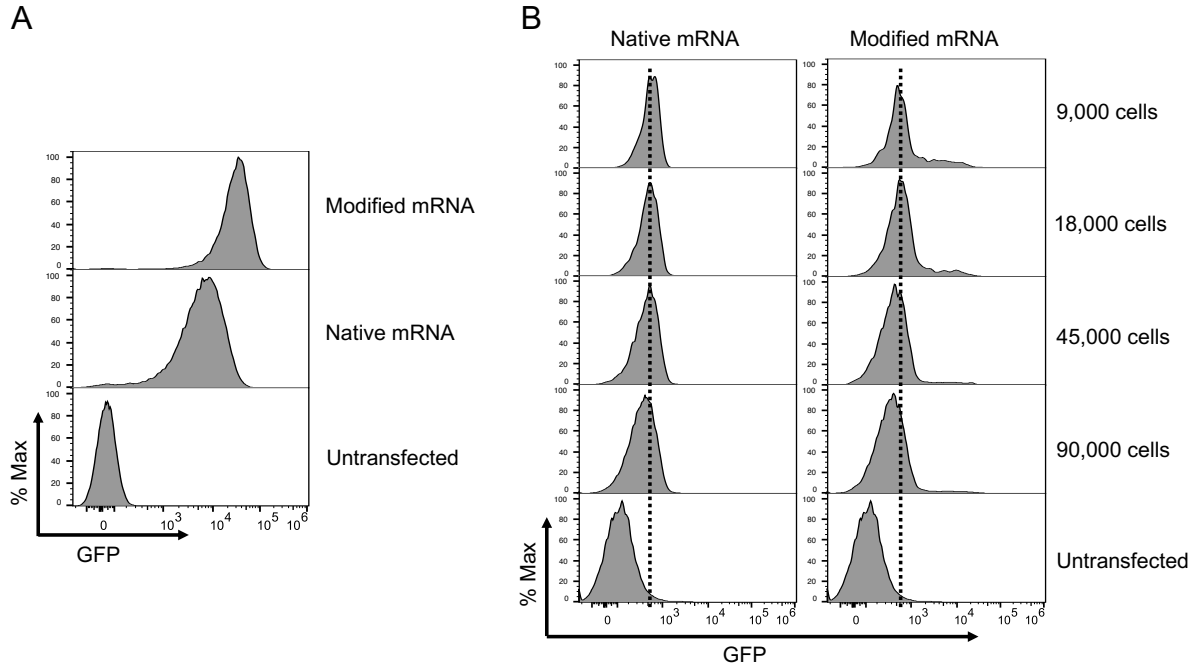

Figure S3. Comparison Of EGFP Expression In A549 Cells Transfected With Native mRNA And Modified mRNA.
A total of 600 ng of native mRNA or modified mRNA synthesized with pseudo-UTP and ACAR cap were used to transfected 90,000 of A549 cells using Lipofectamine 2000 (A) or 9,000 to 90,000 of A549 cells using LNP_{LH} (B). The EGFP expression was tested by flow cytometry after 7 hours.