Supporting Information

Boosting Ionizable Lipid Nanoparticle-mediated In Vivo mRNA Delivery through Optimization of Lipid Amine-Head Group

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Table S1. Primers used for RT-qPCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5’-3’)</th>
<th>Reverse (5’-3’)</th>
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<tbody>
<tr>
<td>GAPDH</td>
<td>GACGGCCGCATCTTTCTTG</td>
<td>GCGCCCAATACGGCCAAATC</td>
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<tr>
<td>EPO</td>
<td>TACCTGCTGGAGGCCA</td>
<td>CACGTTGATGTTCTCGTTCA</td>
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Figure S1. A representative $^1$H NMR spectrum of 114 (A), 214 (B), 314 (C), 414 (D), 514 (E), 614 (F) ionizable lipids in CD$_2$Cl$_2$. 
**Figure S2.** The mRNA encapsulation determined by RiboGreen assay at different weight ratio of iLNP:mRNA (n=2).

**Figure S3.** Colocalization analysis of endosomal escaping from iLNP/mRNA by Pearson’s correlation.
Figure S4. The pKa of iLNPs determined by TNS assay (n=2).

Figure S5. Cell viability of HeLa cells treated with top performance iLNP/mRNA complexes at mRNA concentration of 0.1, 0.2 or 0.4 μg/mL RNA for 24 h (n=3).
Figure S6. Quantification of cellular uptake in HeLa cells following treatment of cells with 114-iLNP in the absence or presence of Apolipoprotein (ApoE, 1 μg/mL) (n=3). Statistical analysis was performed using One-way ANOVA with Tukey test (GraphPad software Prism, Version 6.01) and statistical difference was defined as ***p < 0.001.

Figure S7. Quantification of hEPO mRNA in major organs by RT-qPCR including heart, liver, spleen, lung and kidneys after 6h i.v. injection of 114-iLNP/mhEPO at mRNA dose of 0.5 mg/kg (n=3). GAPDH was utilized as house-keeping gene during RT-qPCR analysis.