

Supplementary Information

Enhanced mRNA and pDNA Delivery via PEGylated Solid Lipid Nanoparticles with an Optimally Balanced Ionizable/Cationic Lipid Content

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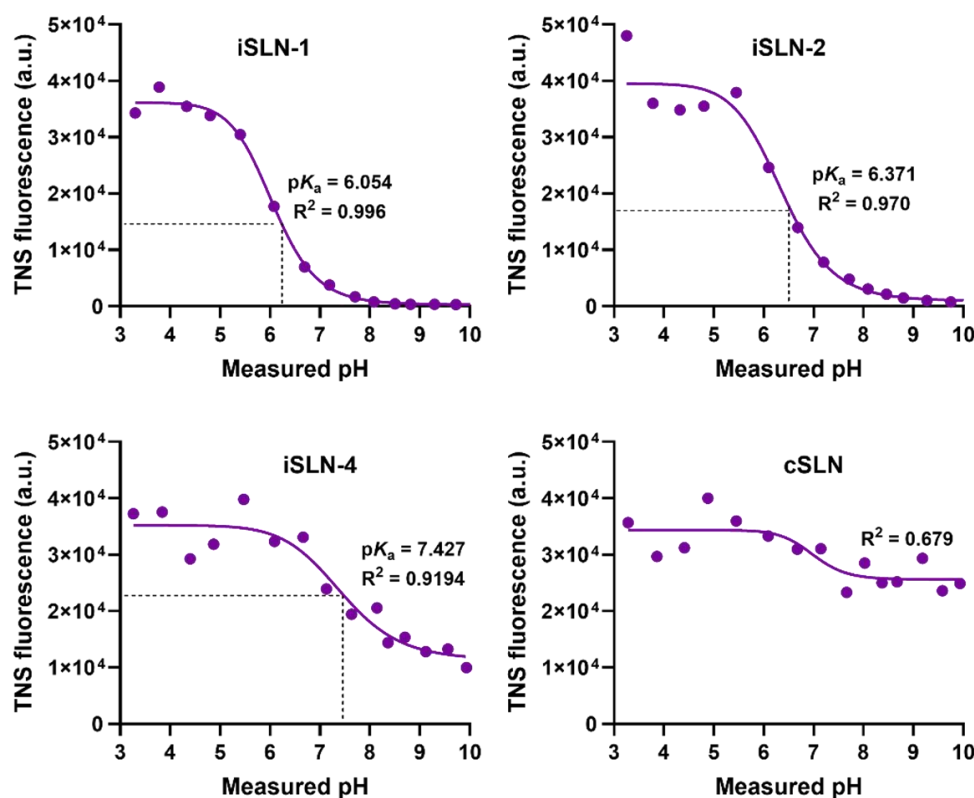


Fig. S1 Representative TNS fluorescence titration curves of iSLN-1, iSLN-2, iSLN-4 and cSLN.

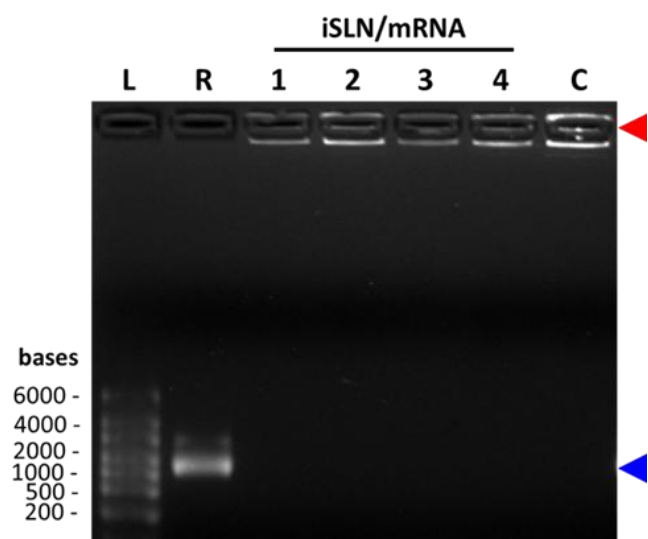


Fig. S2 Gel electrophoresis analysis of iSLN/mRNA and cSLN/mRNA complexes at an N/P ratio of 6. L: ladder (RiboRuler high-range RNA ladder), R: free mRNA, 1-4: iSLN-1/mRNA to iSLN-4/mRNA complexes, C: cSLN/mRNA complexes. Blue and red arrowheads mark the locations of free mRNA and its corresponding complexes, respectively.

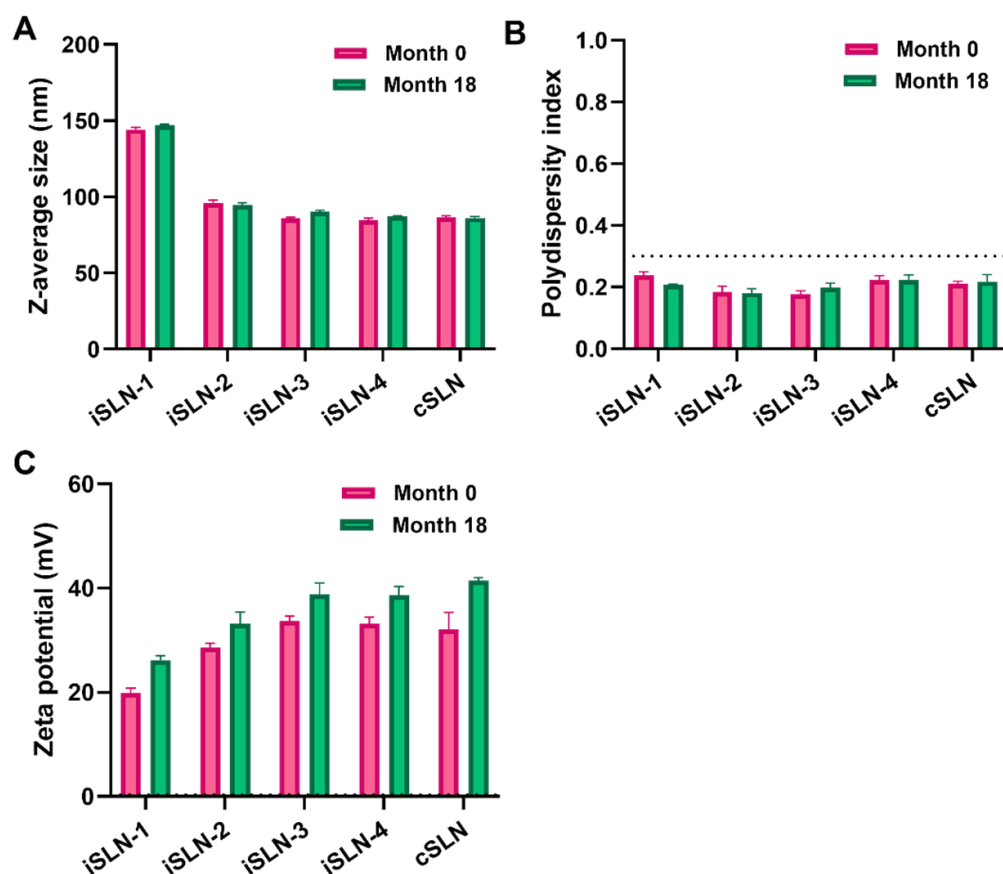


Fig. S3 (A) Z-average size, (B) polydispersity index and (C) zeta potential of bare iSLNs and cSLN measured before and after storage at 4 °C for 18 months. Mean \pm SD ($n = 3$).

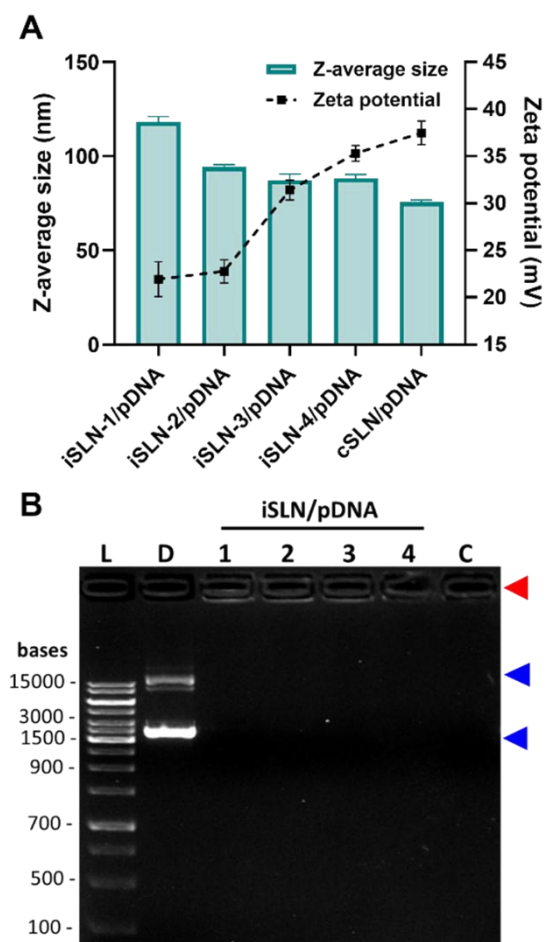


Fig. S4 (A) Z-average size and zeta potential of iSLN/pDNA and cSLN/pDNA complexes. Mean \pm SD ($n = 3$). (B) Gel electrophoresis analysis of iSLN/pDNA and cSLN/pDNA complexes formulated with FLuc pDNA at an N/P ratio of 12. L: ladder (1 kb Plus DNA ladder), D: free pDNA, 1-4: iSLN-1/pDNA to iSLN-4/pDNA complexes, C: cSLN/pDNA complexes. The blue and red arrowheads indicate the location of free pDNA and its corresponding complexes, respectively.

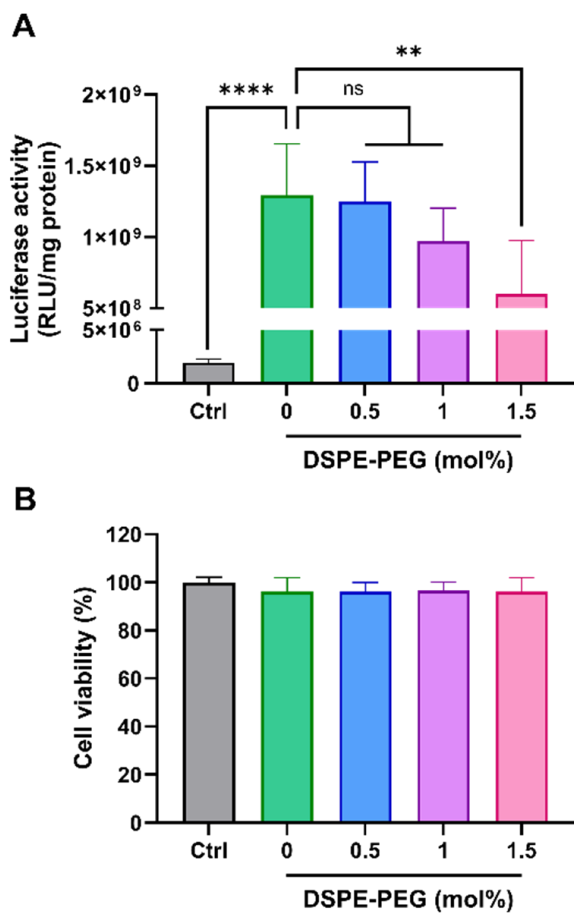


Fig. S5 (A) Luciferase expression levels and (B) viability of HEK293T cells treated for 48 h with PEG-iSLN-3/pDNA complexes with various DSPE-PEG contents (0, 0.5, 1 and 1.5 mol% of total lipid). The complexes were formulated with FLuc pDNA at an N/P ratio of 12. Mean \pm SD ($n = 6$); ** $P < 0.01$; **** $P < 0.0001$; ns: nonsignificant.

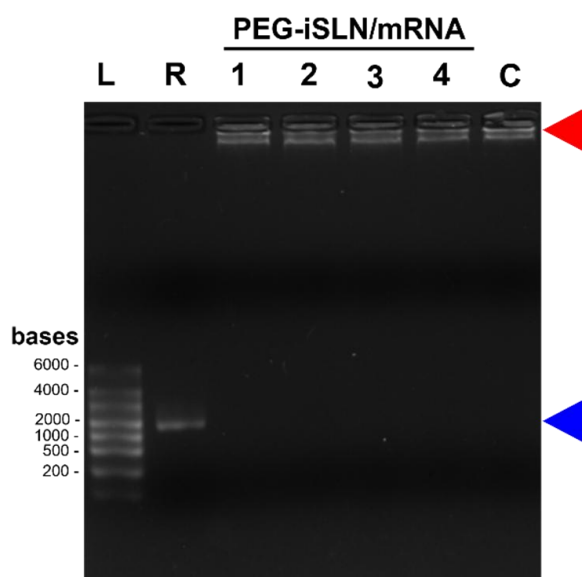


Fig. S6 Gel electrophoresis analysis of PEG-iSLN/mRNA and PEG-cSLN/mRNA complexes formulated with FLuc mRNA at an N/P ratio of 6. L: ladder (RiboRuler high-range RNA ladder), R: free mRNA, 1-4: PEG-iSLN-1/mRNA to PEG-iSLN-4/mRNA complexes, C: PEG-cSLN/mRNA complex. The blue and red arrowheads indicate the location of free mRNA and its corresponding complexes, respectively.

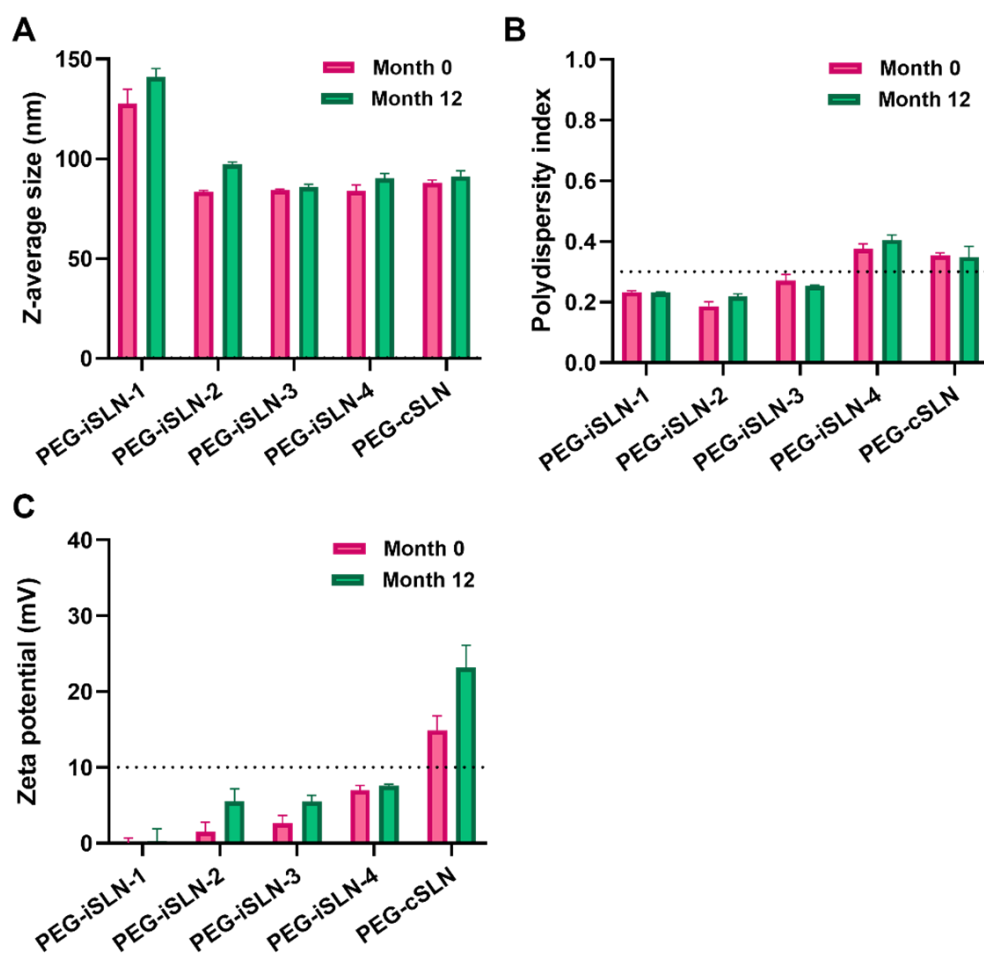


Fig. S7 (A) Z-average size (B) polydispersity index and (C) zeta potential of bare PEG-iSLNs and PEG-cSLN measured before and after storage at 4 °C for 12 months. Mean \pm SD ($n = 3$).

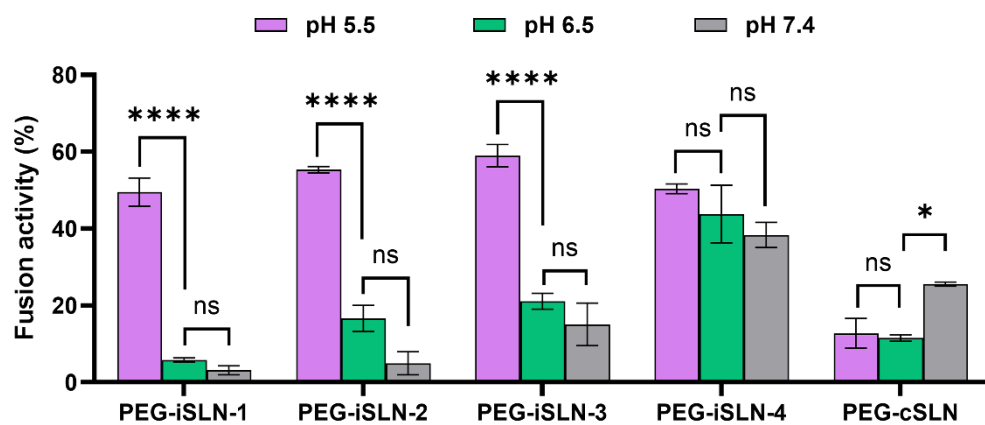


Fig. S8 Membrane fusion activity of bare PEG-iSLN or PEG-cSLN formulations measured at pH 5.5, 6.5 or 7.4. Mean \pm SD ($n = 2$); **** $P < 0.0001$; * $P < 0.05$; ns: nonsignificant.

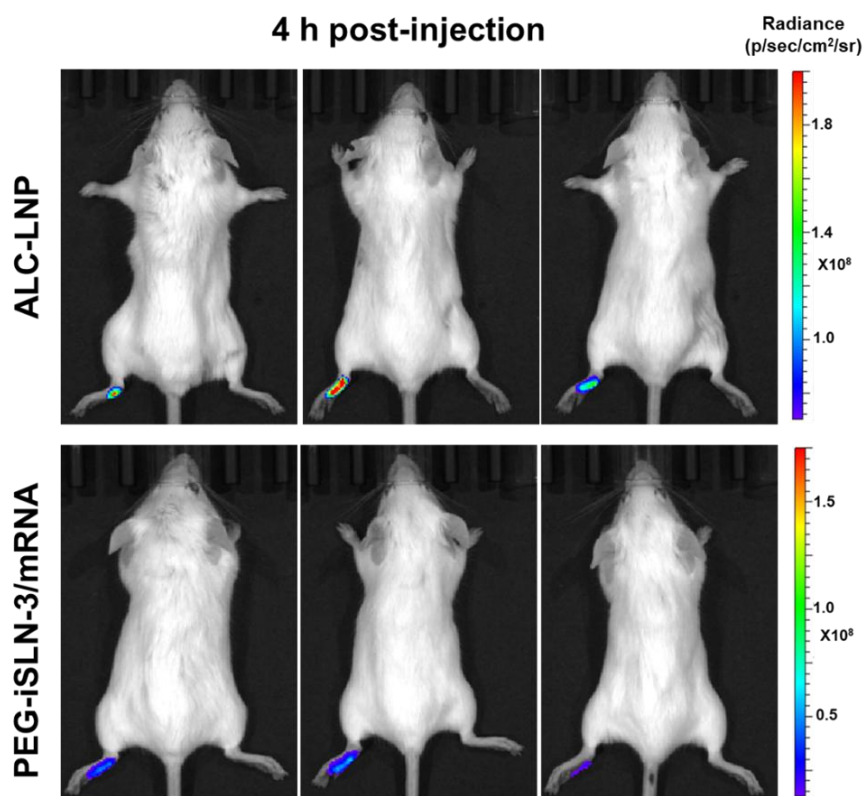


Fig. S9 Whole-body bioluminescence images of BALB/c mice taken at 4 h after footpad subcutaneous injection of ALC-LNP or PEG-iSLN-3/mRNA complexes at the same dose (1 μ g of FLuc mRNA/mouse).

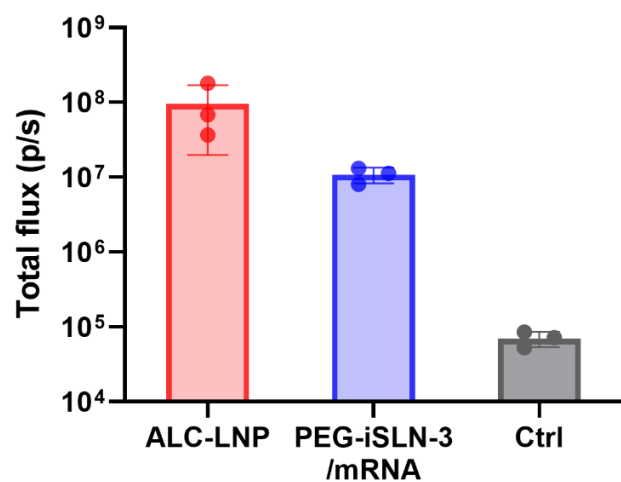


Fig. S10 Total flux values of the hind leg muscle (injection site) of BALB/c mice following the intramuscular administration of ALC-LNP or PEG-iSLN-3/mRNA complexes (1 μ g of FLuc mRNA/mouse). Naive mice that received only luciferin solution were used as a mock control (Ctrl). Mean \pm SD ($n = 3$).