Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary material

Ionic liquid-coated lipid nanoparticles increase siRNA uptake into CNS targets

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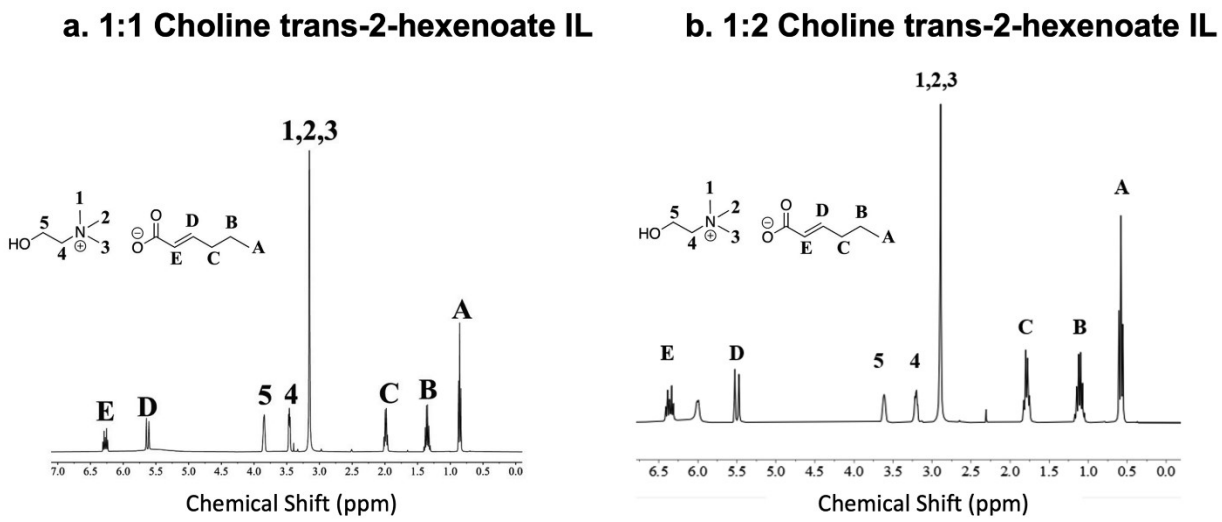
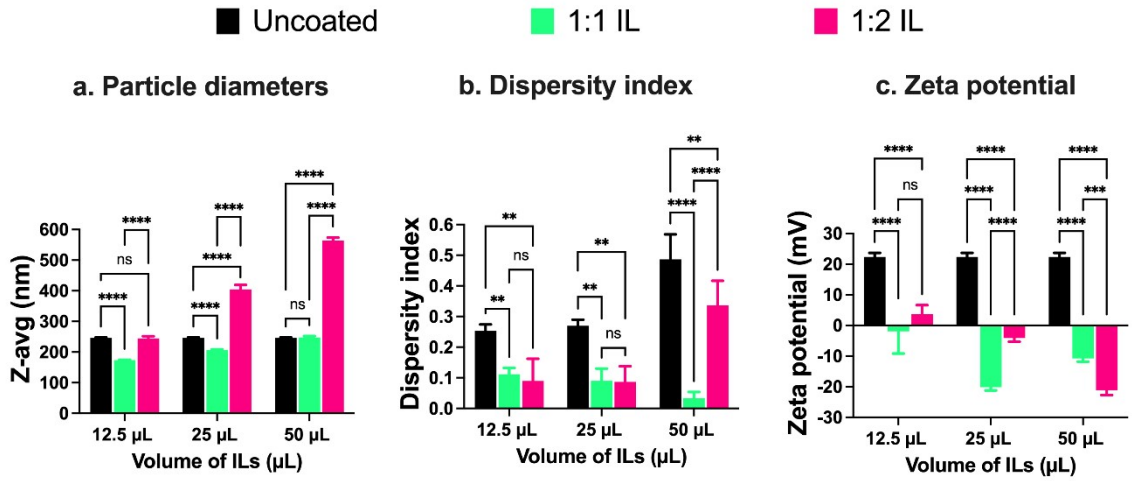
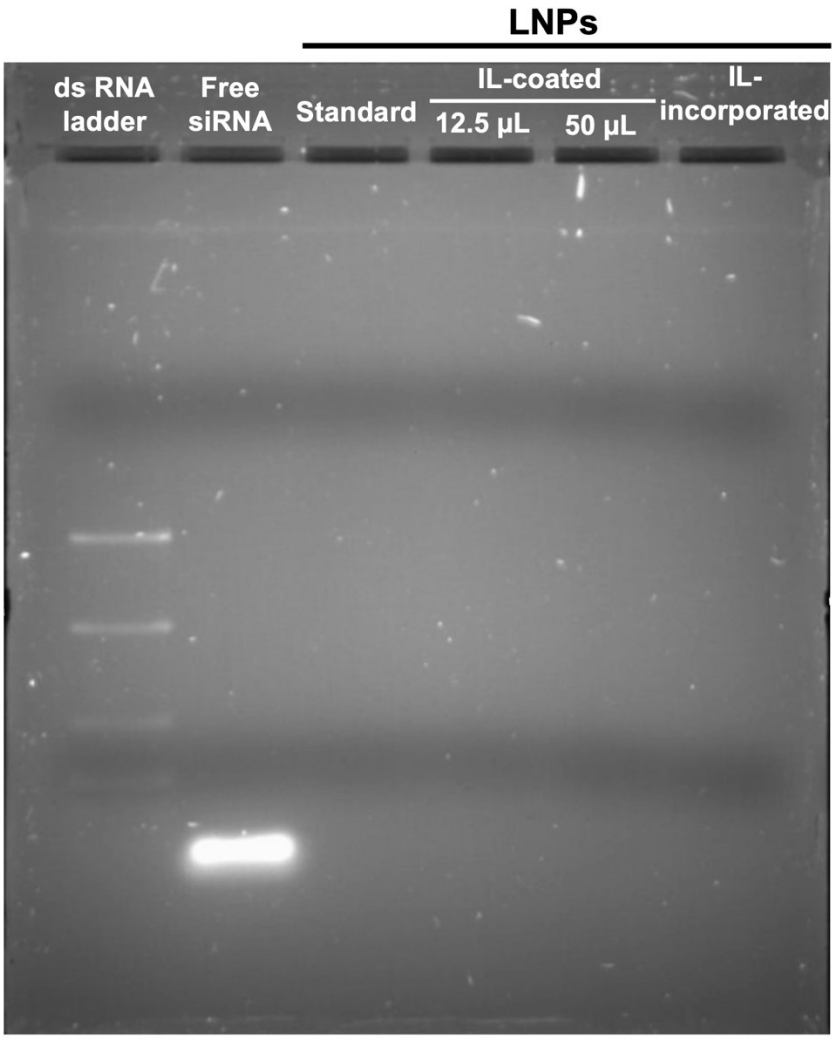


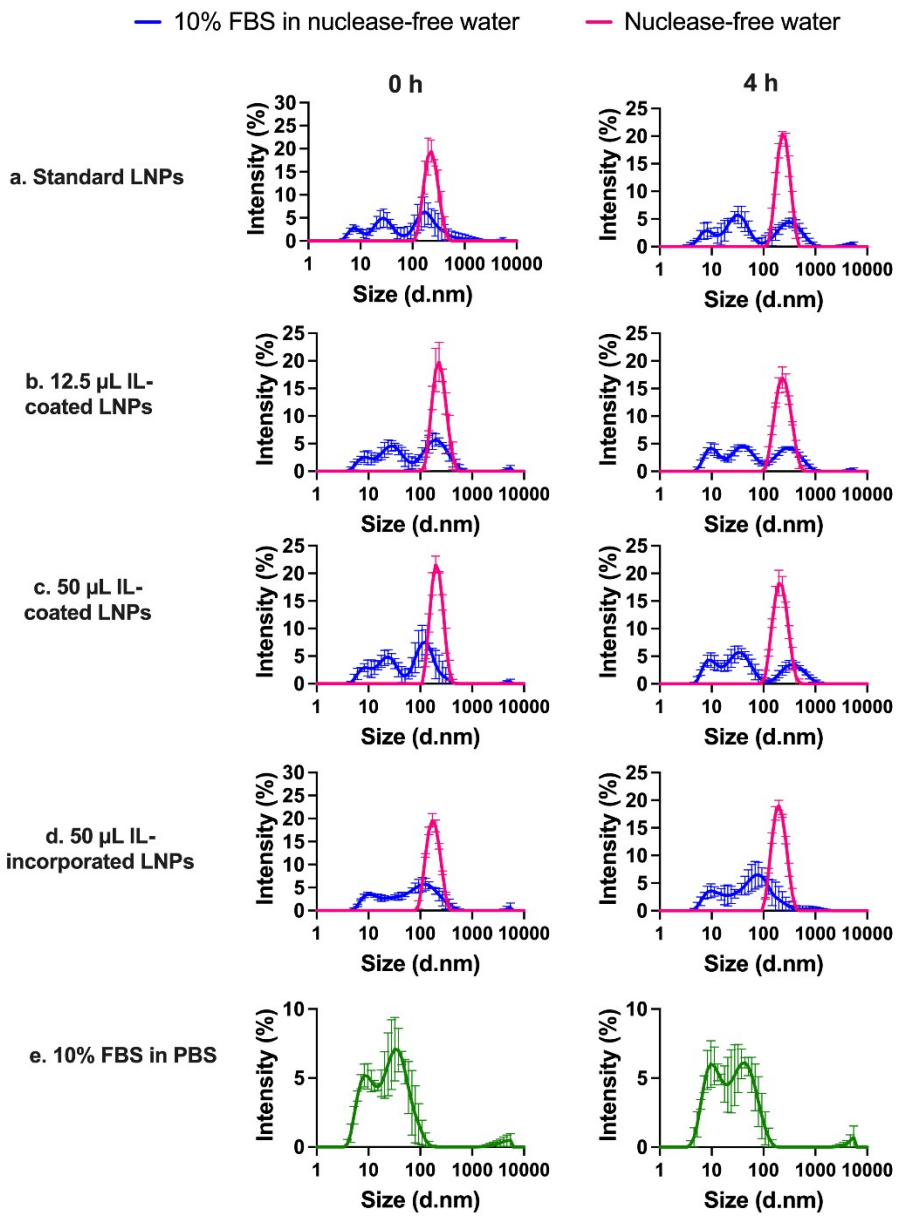
Supplementary figure 1. ^{1}H NMR of 1:1 (a) and 1:2 (b) choline trans-2-hexenoic acid. [9, 10]



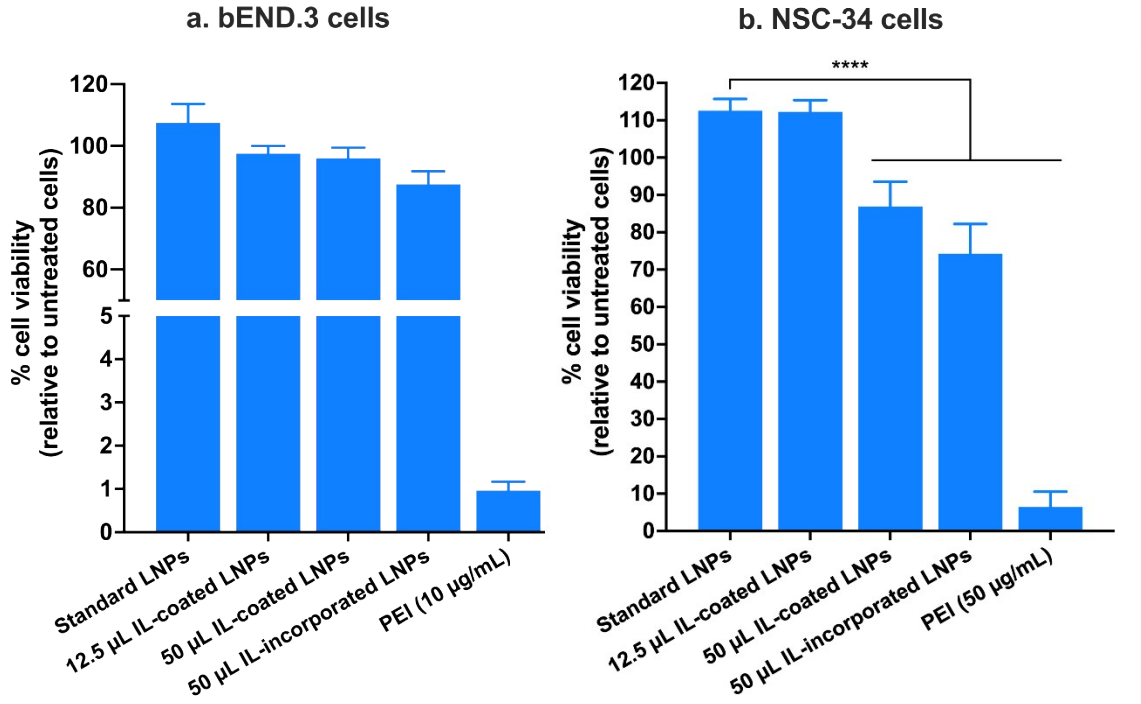
Supplementary figure 2. Particle diameters (a), dispersity indices (b), and zeta potential (c) of 1:1 and 1:2 IL-coated siRNA-LNPs measured using dynamic light scattering. siRNA-LNPs (400 nM) were initially prepared in DI water and further diluted 10*x* using DI water. 12.5, 25, and 50 µL of 1:1 or 1:2 choline trans-2-hexenoate IL were added to the siRNA-LNPs followed by bath sonication for 60 minutes. Z-average particle diameters, dispersity indices, and zeta potential were measured using a Malvern Zetasizer Pro. Statistical analysis was done using two-way ANOVA with Tukey's multiple comparisons test. Data are presented as mean ± SD of n=3 measurements. **p<0.01, ****p<0.0001 and ns: nonsignificant.



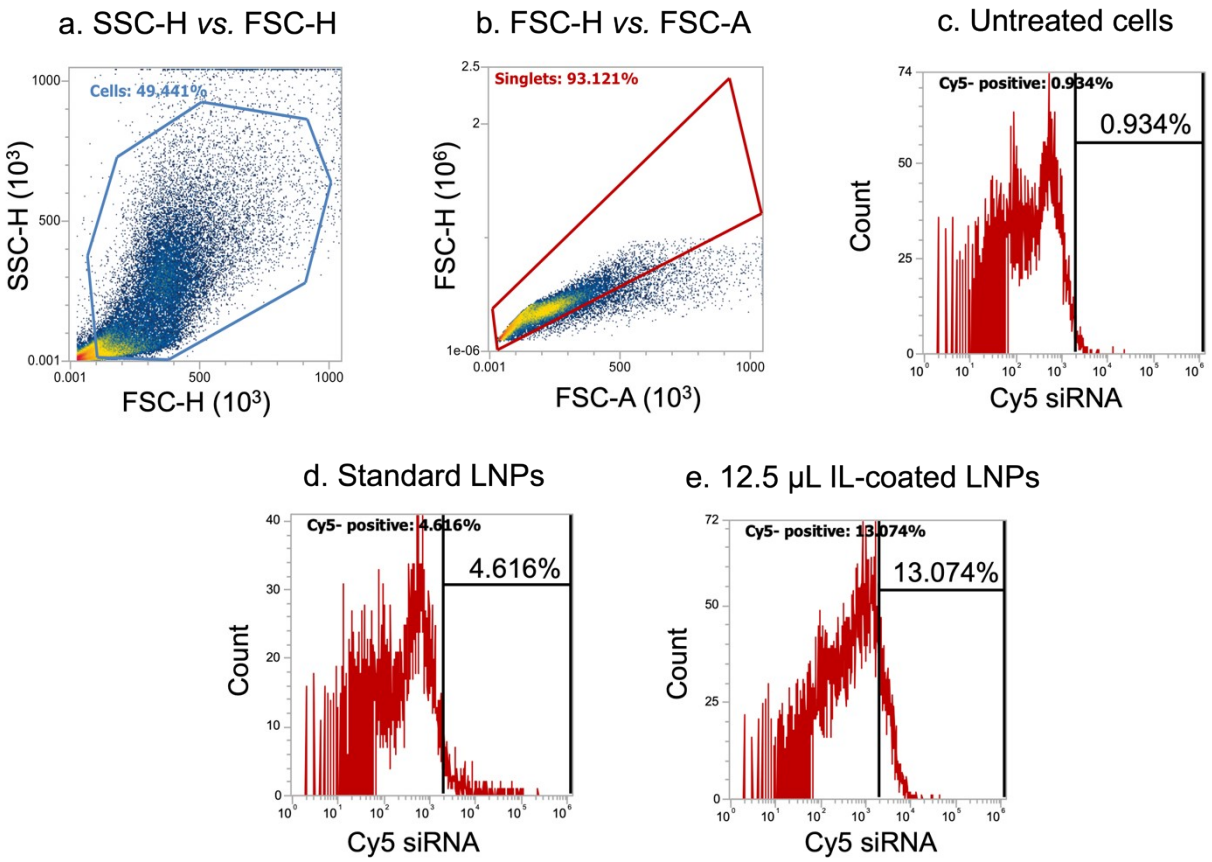
Supplementary Figure 3. Agarose gel electrophoresis to confirm the encapsulation of siGFP in LNPs. One µg of naked siGFP and siGFP-loaded LNP formulations were loaded in a 2% agarose gel, electrophoresed, and visualized under UV light.



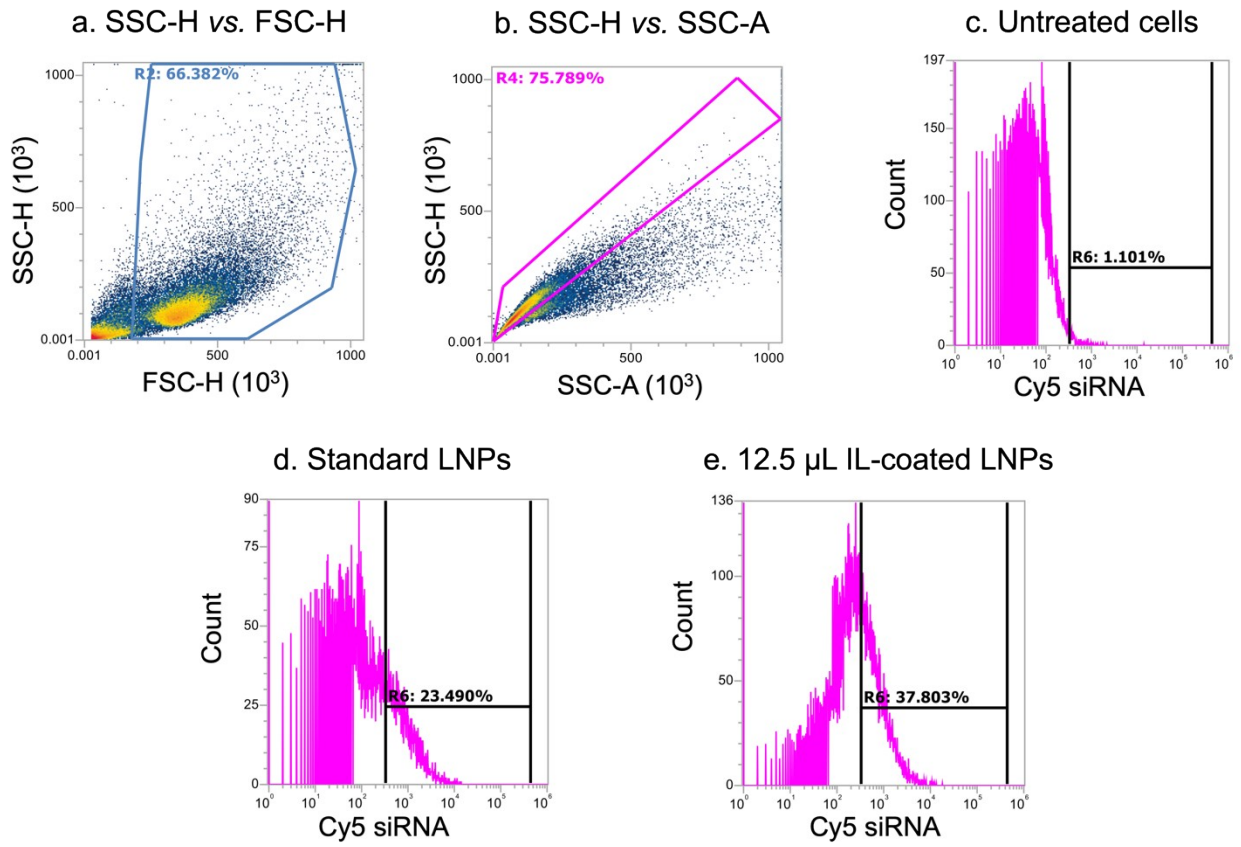
Supplementary Figure 4. Intensity size distribution plots demonstrating the colloidal stability of standard (a), IL-coated (b and c), and IL-incorporated LNPs (d) with/without 10% FBS. siGFP-LNPs (400 nM) were prepared in 10 mM citrate buffer and diluted to a final siRNA concentration of 50 nM using nuclease-free water (pink) (**a**). Further, LNPs were coated with 12.5 (**b**) or 50 µL (**c**) of 1:1 choline trans-2-hexenoate IL. IL-incorporated LNPs were prepared by replacing PEG-DMG with IL (**d**). LNPs supplemented with 10% FBS (blue) were measured by DLS at 0 and 4 h to determine their serum stability. A blank sample of 10% FBS in nuclease-free water (green) accounted for the serum protein-mediated scattering **(e)**.



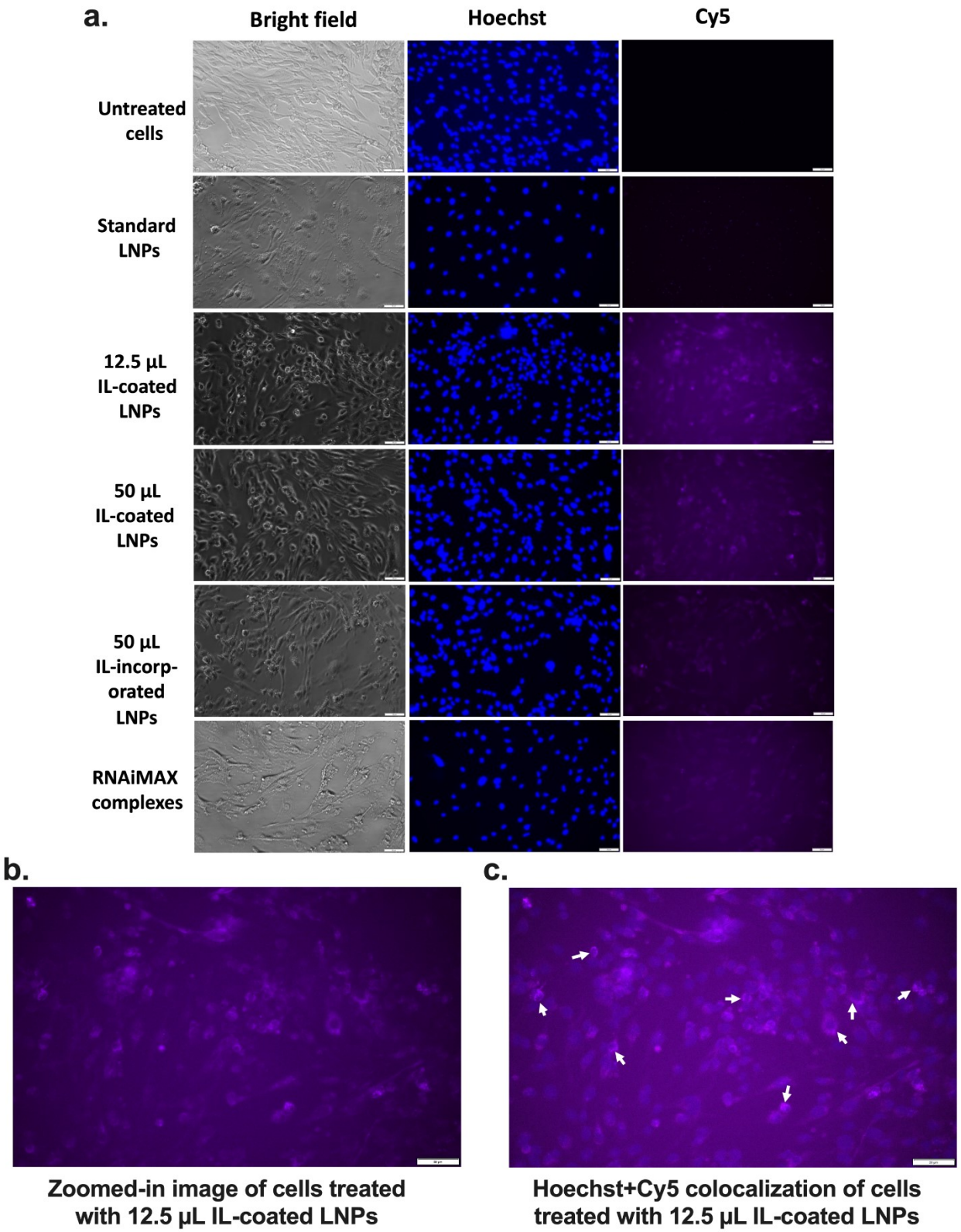
Supplementary Figure 5. Cytocompatibility of IL-coated LNPs and IL-incorporated LNPs with a mouse brain endothelial cell line, bEnd.3 (a) and NSC-34 motor neuron-enriched, embryonic mouse spinal cord cell line fused with mouse neuroblastoma (b). Cells were incubated with standard LNPs, IL-coated LNPs, and IL-incorporated LNPs containing 50 nM siGFP for four hours. Untreated cells and cells treated with PEI (10/50 µg/mL) were used as negative and positive controls, respectively. Cell viability was determined 24 h-post-transfection using a CellGlo luminescence viability assay and the data were normalized to untreated cells. Data represents mean + SD (n=6). ****$p<0.0001$.



Supplementary figure 6. Flow gating strategy and flow plots for the cellular uptake of standard and IL-coated LNPs into bEnd.3 BECs. bEnd.3 BECs were treated for 4 h with standard LNPs or IL-coated LNPs loaded with 50 nM Cy5 siRNA in complete growth medium in a humidified incubator at 37 °C and 5% CO_2. Untreated cells were used as a negative control. SSC-H *vs.* FSC-H (a) and FSC-H *vs.* FSC-A (b) plots of untreated cells were used to gate the main cell population and the monodisperse cell population, respectively. The autofluorescence of untreated cells was gated out as shown in (c). Histogram plots for cells treated with standard LNPs (d) and 12.5 µL IL-coated LNPs (e) depict positive shifts in the uptake of Cy5 siRNA by cells.



Supplementary figure 7. Flow gating strategy and flow plots for the cellular uptake of standard and IL-coated LNPs containing Cy5 siRNA into NSC-34 motor neurons. NSC-34 motor neurons were treated for 4 h with standard LNPs and IL-coated LNPs loaded with 50 nM Cy5 siRNA in the differentiation medium in a humidified incubator at 37 °C and 5% CO_2. Untreated cells were used as a negative control. SSC-H *vs.* FSC-H (a) and SSC-H *vs.* SSC-A (b) plots of untreated cells were used to gate the main cell population and the monodisperse cell population, respectively. The autofluorescence of untreated cells was gated out as shown in (c). Histogram plots for cells treated with standard LNPs (d) and 12.5 µL IL-coated LNPs (e) depict positive shifts in the uptake of Cy5 siRNA by cells.



Supplementary Figure 8. Cellular uptake of Cy5 siRNA containing standard, IL-coated, and IL-incorporated LNPs into bEnd.3 cells using fluorescence microscopy. bEnd.3 cells were incubated for 4

hours with the indicated samples containing 50 nM Cy5 siRNA-LNPs. Cells were stained with 10 µg/mL Hoechst for staining the nuclei in viable cells. Scale bar = 50 µm. Cells were imaged using an Olympus IX 73 epifluorescent microscope to detect Cy5 signals at excitation and emission wavelengths of 651 nm and 670 nm. In contrast, Hoechst signals were detected at excitation and emission wavelengths of 350 nm and 461 nm, respectively. Cy5 and Hoechst signals were false-colored as purple and blue, respectively. Images are representative of n=4 independent wells (**a**). Zoomed-in image of cells treated with 12.5 µL 1:1 IL-coated LNPs (**b**) and colocalization of Hoechst (blue) + Cy5 (purple) to show cytosolic uptake of Cy5 siRNA in cells treated with 12.5 µL IL-coated LNPs (white arrows indicate colocalization of signals) **(c)**.

Supplementary table 1. Comparison of particle diameters, dispersity indices, and zeta potential of standard and IL-re-engineered LNPs

Samples	Particle diameters (nm)	Dispersity index	Zeta potential (mV)
Standard LNPs	214.2 ± 3.7	0.07 ± 0.01	18.9 ± 1.4
12.5 µL 1:1 IL-coated LNPs	173 ± 0.8	0.11 ± 0.02	-1.9 ± 7.2
12.5 µL 1:2 IL-coated LNPs	244.8 ± 6.8	0.09 ± 0.07	3.7 ± 3.0
IL-incorporated LNPs	218 ± 10.5	0.06 ± 0.01	-2.5 ± 0.3