Supplementary Method 1: Formulation of siRNA-loaded lipid nanoparticles (LNPs)

LNPs were formulated by mixing the ionizable lipidoid 306O_{i10},[22,25] cholesterol (Millipore Sigma, Burlington, MA), 1, 2-distearoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Alabaster, AL), and 1, 2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (C14PEG2000) (Avanti Polar Lipids) at a molar ratio of 50: 38.5: 10: 1.5, as previously described.[22–25] The lipidoid 306O_{i10} was synthesized by Michael addition reaction using an isodecyl acrylate to 3,3-diamino-N-methyldipropylamine stoichiometric ratio of 4:1, as previously described.[22,25] To yield a lipid solution composed of 90% (v/v) ethanol and 10% citrate buffer, additional ethanol and 10 mM sodium citrate buffer (pH 4.2) were added.[22] The siRNA solution was prepared by diluting siRNA in 10 mM sodium citrate to yield a final weight lipidoid: siRNA of 5:1.[22] Equal volumes of lipid solution and siRNA solution were rapidly mixed together using a vortex mixer to form nanoparticles.[24] LNPs were further diluted in phosphate-buffered saline (PBS) and then dialyzed against PBS for 90 minutes in 3500 MWCO cassettes (Pierce/Thermo Scientific, Rockford, IL) to remove ethanol.[24]

References

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