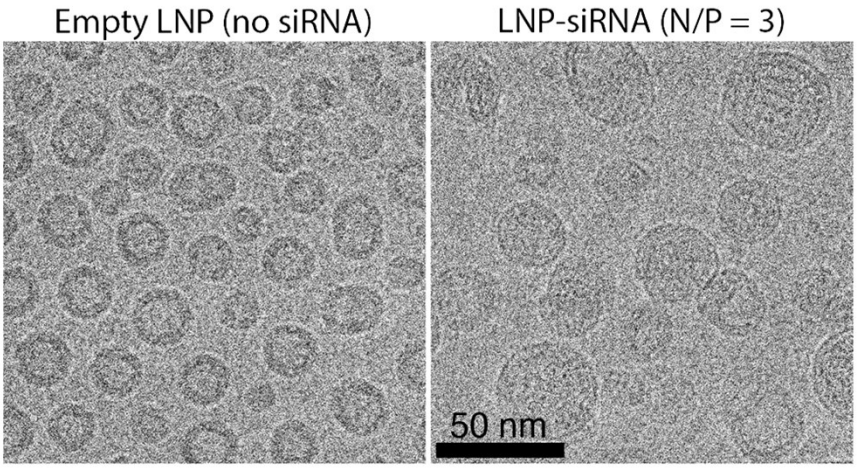
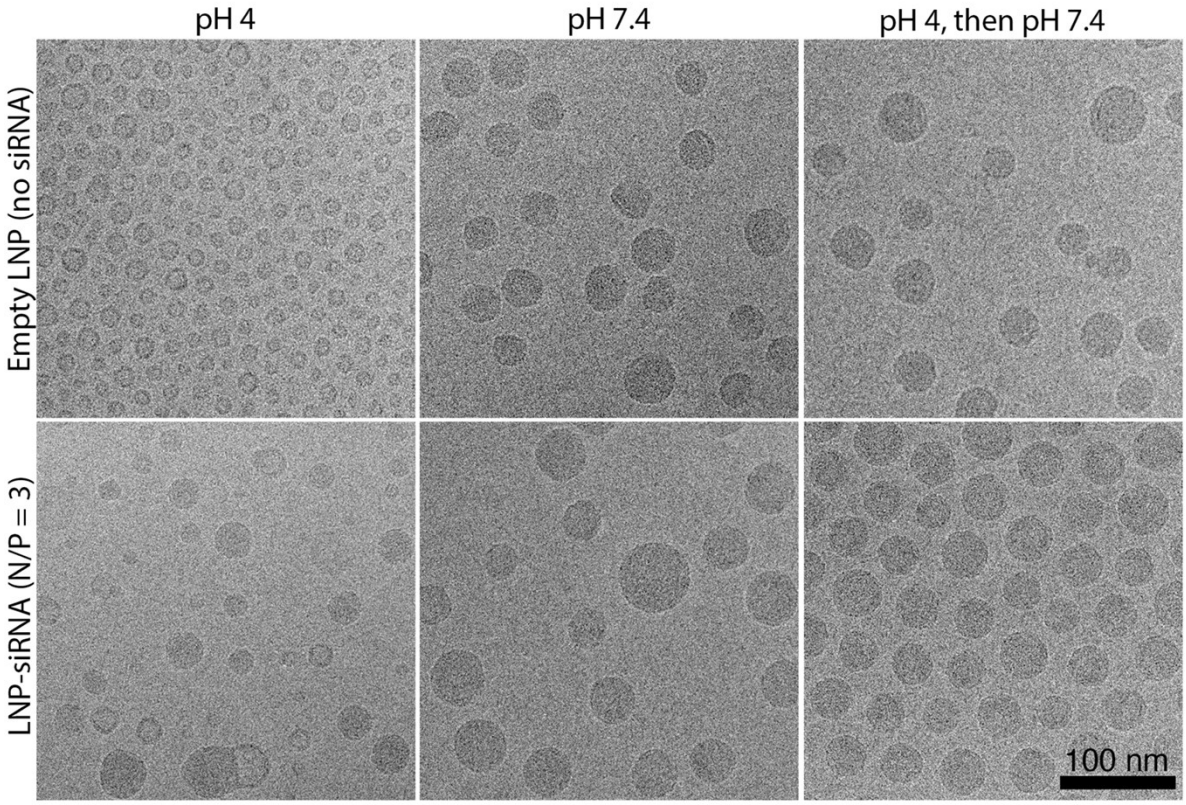
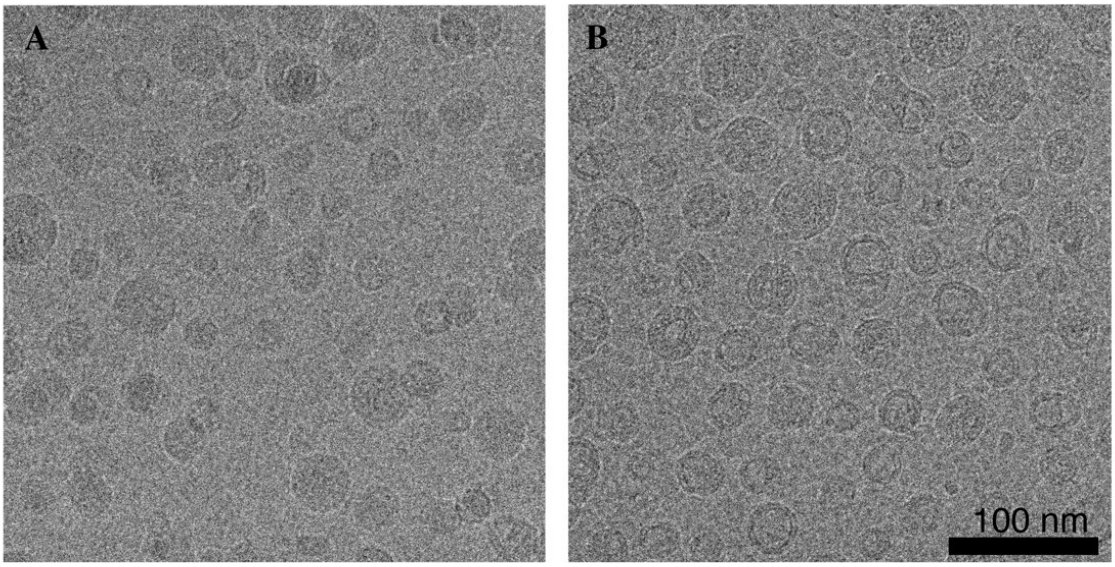
Electronic Supplementary Material (ESI) for Nanoscale.



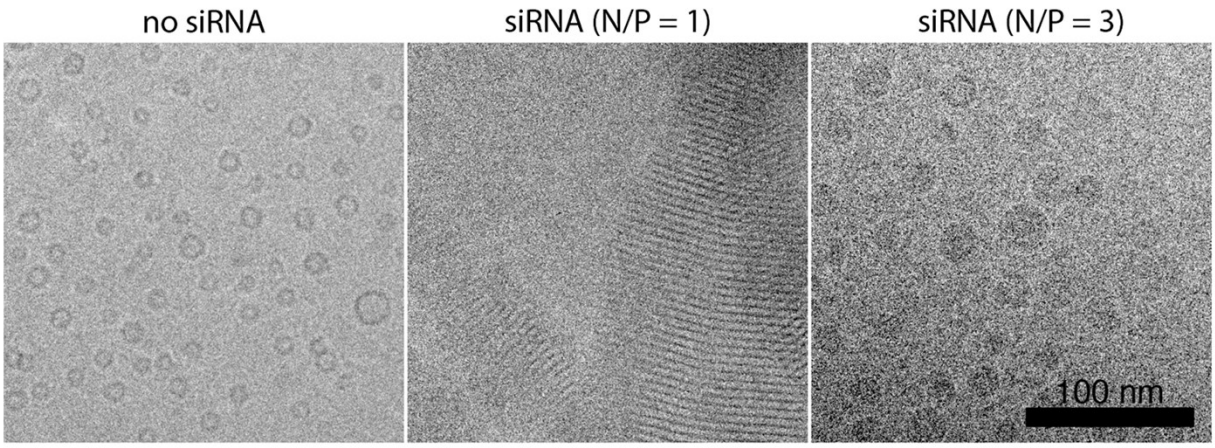
Supporting Figure 1. Morphology of empty LNP systems at pH 4 is different from LNP-siRNA (N/P = 3). LNP composed of KC2/DSPC/Chol/PEG-lipid (50/10/38.5/1.5 mol%) were formulated without siRNA (empty LNP) or with siRNA (N/P = 3) at pH 4 and dialysed into the same pH 4 buffer to remove solvent. The resulting samples were concentrated and imaged by cryo-TEM at a magnification of 96,000x. *Scale bar = 50 nm.*



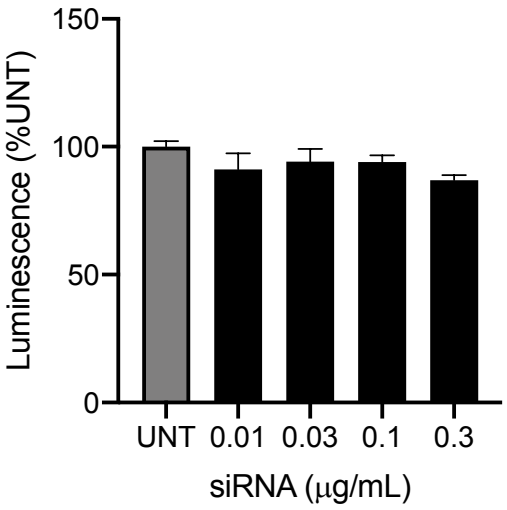
Supporting Figure 2. LNP metamorphosis is dictated by deprotonation of the ionizable cationic lipid (rather than the presence of ethanol). LNP composed of KC2/DSPC/Chol/PEG-lipid (50/10/38.5/1.5 mol%) were formulated with (N/P = 3) and without siRNA at pH 4 and dialysed directly into pH 4 buffer or pH 7.4 PBS. An aliquot from the pH 4 formulation (after removal of ethanol) was then dialysed against PBS. The three resulting samples (per formulation) were concentrated and imaged by cryo-TEM. *Scale bar = 100 nm.*



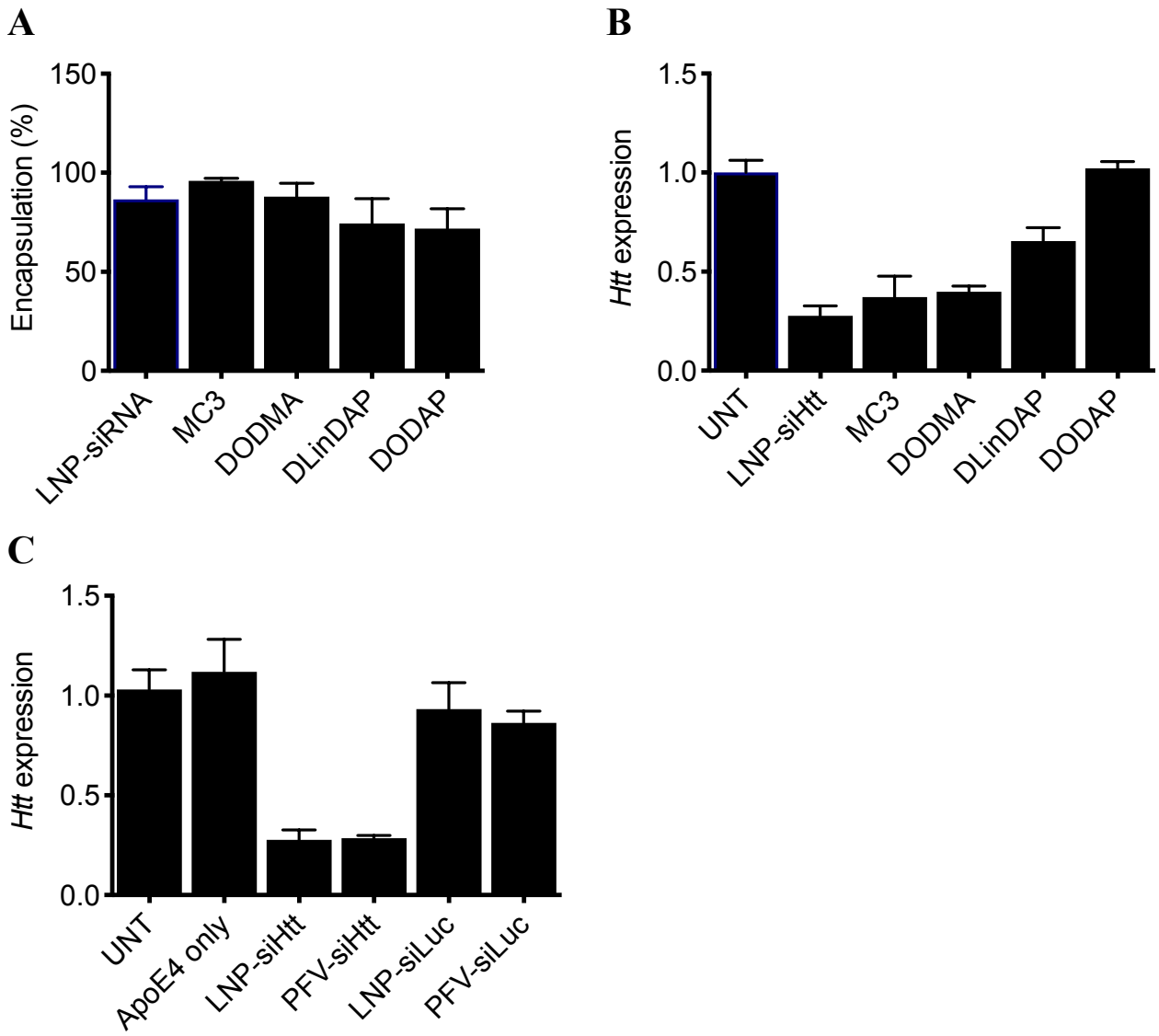
Supporting Figure 3. Single- and two-phase particles display essentially similar morphology at pH 4. LNP composed of KC2/DSPC/Chol/PEG-lipid (50/10/38.5/1.5 mol%) were formulated with siRNA at (N/P = 3) at pH 4. (A) For two-phase systems, the ethanol was removed by dialysis. (B) Single-phase systems were prepared by combining PFV in pH 4 buffer with siRNA in the same buffer. Both samples were concentrated and analysed by cryo-TEM. *Scale bar = 100 nm*.



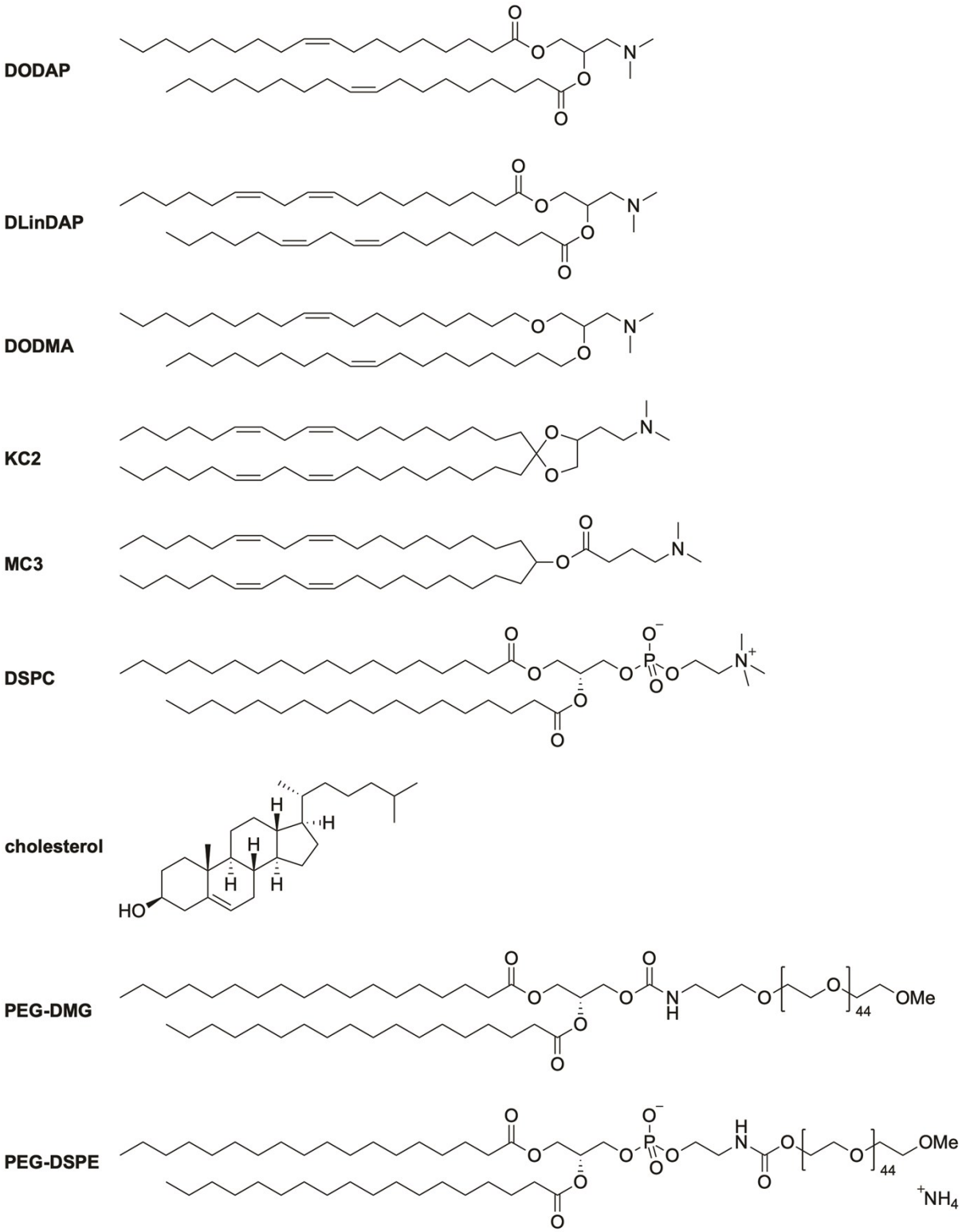
Supporting Figure 4. Particles containing high amounts of KC2 prepared at N/P = 1 form stacked lamellar structures, but spherical structures at N/P = 3. LNP composed of KC2 /PEG-lipid (98.5/1.5 mol%) were formulated without siRNA at pH 4 and dialysed directly into pH 4 buffer. Single-phase mixing was used to combine the particles with siRNA at N/P ratios of 1 and 3. The three resulting samples were concentrated and imaged by cryo-TEM. *Scale bar = 100 nm.*



Supporting Figure 5: Treatment of primary cortical neurons with PFV-siRNA is well-tolerated. Luciferase-based viability assessment of GFP-expressing primary cortical neurons 72h after treatment with PFV-siRNA against GFP. Data are normalized to % untreated cells and presented as mean ± SEM (n=4 wells/condition).



Supporting Figure 6. PFV are encapsulation competent and effective for knockdown of an endogenous gene target *ex vivo*. PFV composed of ionizable lipid/DSPC/Chol/PEG-lipid (50/10/38.5/1.5 mol%) were prepared at pH 4 and combined with nucleic acid on the benchtop in an Eppendorf tube. (A) RiboGreen-based measurement of the encapsulation of each PFV formulation. (B) Knockdown of murine huntingtin (*Htt*) in primary cortical neurons following treatment with preformed LNP (LNP-si*Htt*), or PFV-si*Htt* immediately prior to treatment. PFV were composed of MC3, DODMA, DLinDAP, or DODAP. (C) Knockdown of murine huntingtin (*Htt*) in primary cortical neurons following treatment with preformed LNP (LNP-si*Htt*) or PFV-si*Htt*, control siRNA (against firefly luciferase, siLuc), ApoE4 only or untreated cells. Results represent mean ± standard deviation (n=3 independent experiments).



Supporting Figure 7. Structures of the lipids used in this study.