Supplementary Information

Lipid nanoparticle formulations for enhanced co-delivery of siRNA and mRNA

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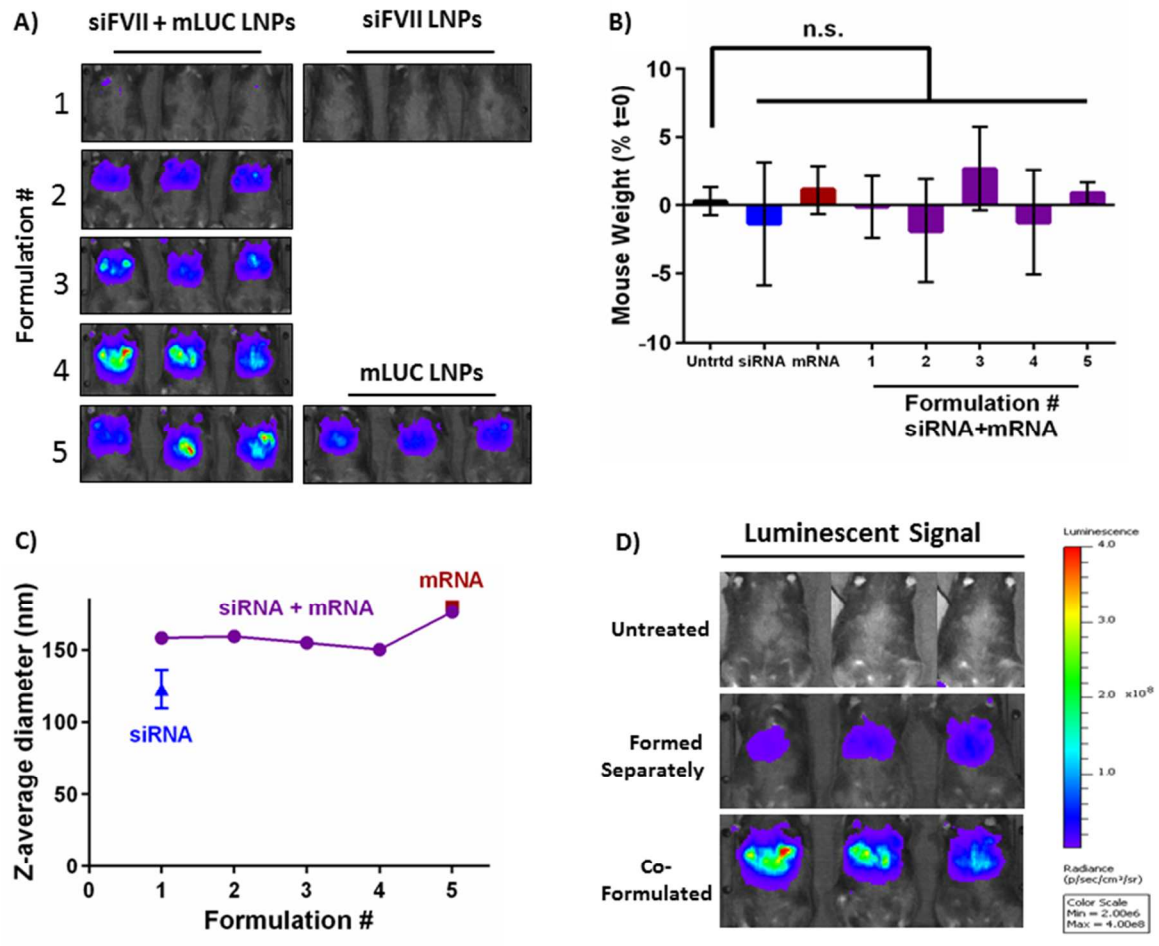

Figure S1. A) LNP formulation 4 resulted in the highest luciferase expression in mice. The vast majority of signal was produced in the liver and spleen. IVIS images were taken 6 hours after tail vein injection of LNPs with 0.5 mg/kg mRNA encoding firefly luciferase (mLUC) and/or 0.03 mg/kg siRNA against FVII (siFVII). (n = 3) **B)** The weight of treated mice did not change compared to untreated mice 2 days following treatment. (n = 3 – 4) **C)** LNP z-average diameter in formulations 1-5 was measured using dynamic light scattering. (n = 3 technical replicates) **D)** LNPs co-formulated with siRNA and mRNA produced the highest luminescent signal in the abdomen of mice. Whole mice were imaged 6 hours after tail vein injection of LNPs in formulation 4 with 0.5 mg/kg mLUC and/or 0.03 mg/kg siFVII (n = 3). Error bars represent s.d.

	siRNA only	mRNA only	siRNA + mRNA				
Formulation #	**1**	**5**	**1**	**2**	**3**	**4**	**5**
Zeta Potential (mV)	-2.91 ± 0.54	-0.78 ± 0.55	-1.11 ± 0.73	-1.92 ± 0.20	-1.71 ± 1.29	0.65 ± 0.52	0.17 ± 0.84

Table S1. The zeta potential was approximately neutral for the five LNP formulations made with siRNA and mRNA. These formulations correspond to those described in Fig. 2. Measurements taken in PBS at pH 7.4. Values represent average ± standard deviation. (n = 3 technical replicates).

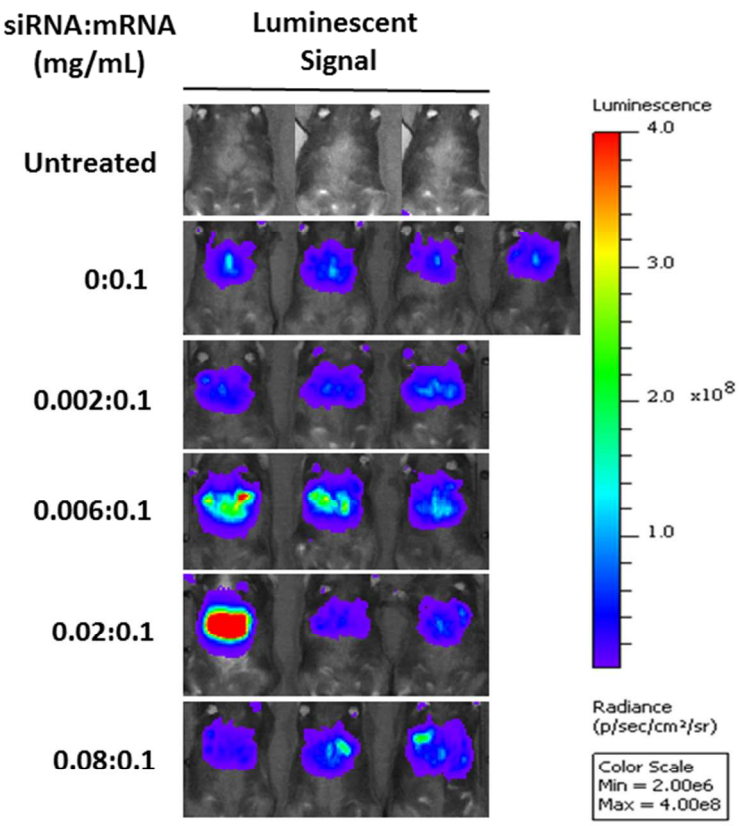

Figure S2. The addition of "helper" siRNA to LNPs loaded with luciferase encoding mRNA improved mRNA delivery for the siRNA: mRNA weight ratio of 0.006 : 0.1. LNPs were formulated using Formulation 4 keeping the mLuc concentration constant (0.1 mg/mL) and changing the siRNA concentration (0 – 0.08 mg/mL). All mice received 0.5 mg/kg mLuc. Mice were imaged 6 hours after tail vein injection. (n = 3 - 4).

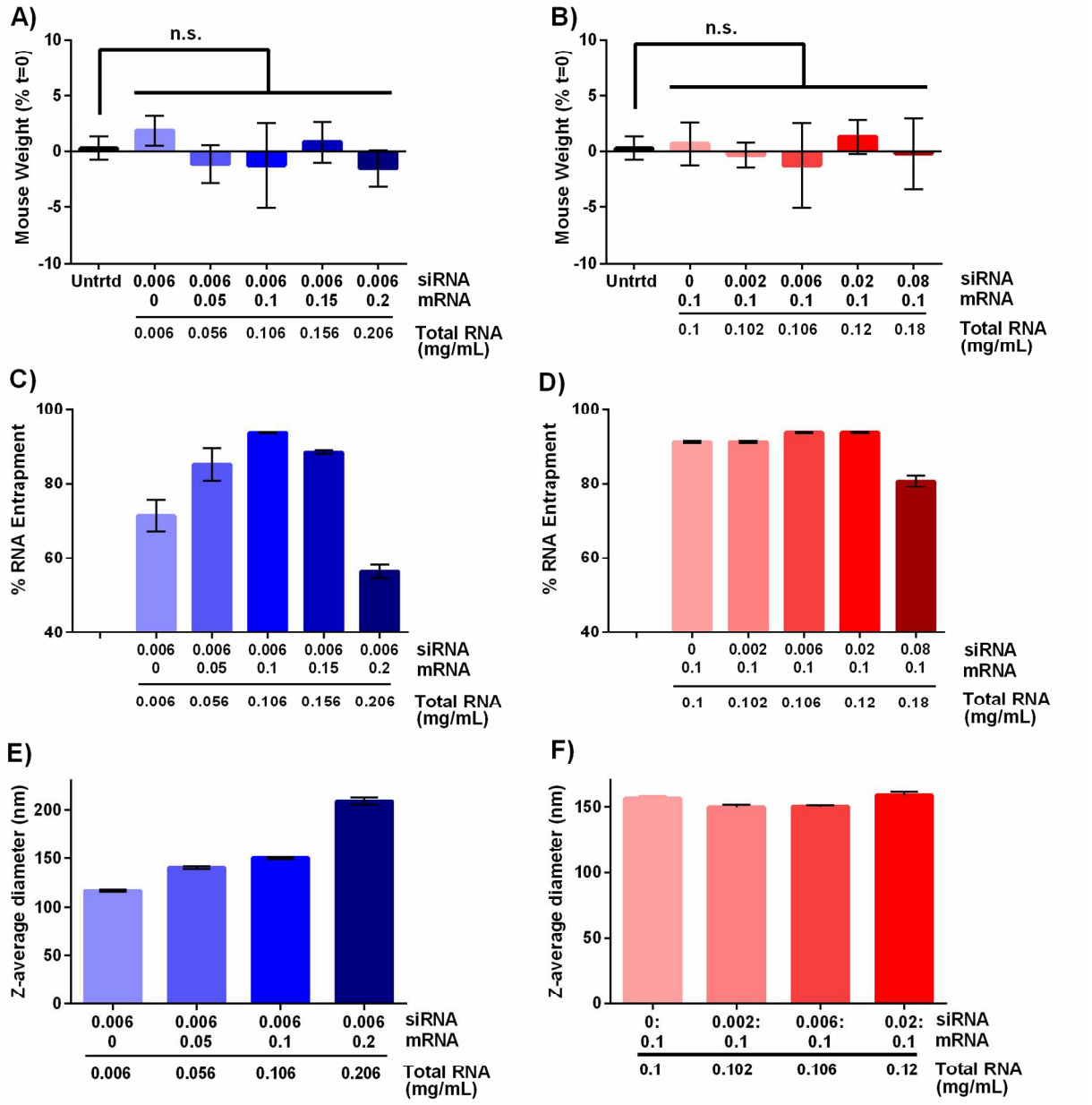

Figure S3. For **A, C, & E** LNPs were formulated using Formulation 4 with a constant siRNA concentration (0.006 mg/mL) and varied mRNA concentrations (0.05-0.15 mg/mL). All mice were dosed with 0.03 mg/kg siFVII. For **B, D, & F** LNPs were formulated using Formulation 4 with a constant mRNA concentration (0.1 mg/mL) and varied siRNA concentration (0 – 0.08 mg/mL). All mice were dosed with 0.5 mg/kg mLuc. **A, B)** LNPs did not induce weight change compared to untreated mice. (n = 3) **C, D)** RNA entrapment in LNPs changed with total RNA concentration during formulation. (n = 3 technical replicates) **E, F)** The z-average diameter of the LNPs ranged from 100 nm – 200 nm. (n = 3 technical replicates). In all panels, error bars represent s.d.

	mRNA added to siRNA					siRNA added to mRNA				
siRNA (mg/mL)	0.006	0.006	0.006	0.006	0.006	0	0.002	0.006	0.02	0.08
mRNA (mg/mL)	0	0.05	0.1	0.15	0.2	0.1	0.1	0.1	0.1	0.1
N:P Ratio	8.3	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4

Table S2. The nitrogen to phosphate (N:P) ratio was constant for formulations including varied total amounts of RNA. LNPs were formulated at the concentrations shown above using formulation #4 from Table 1.

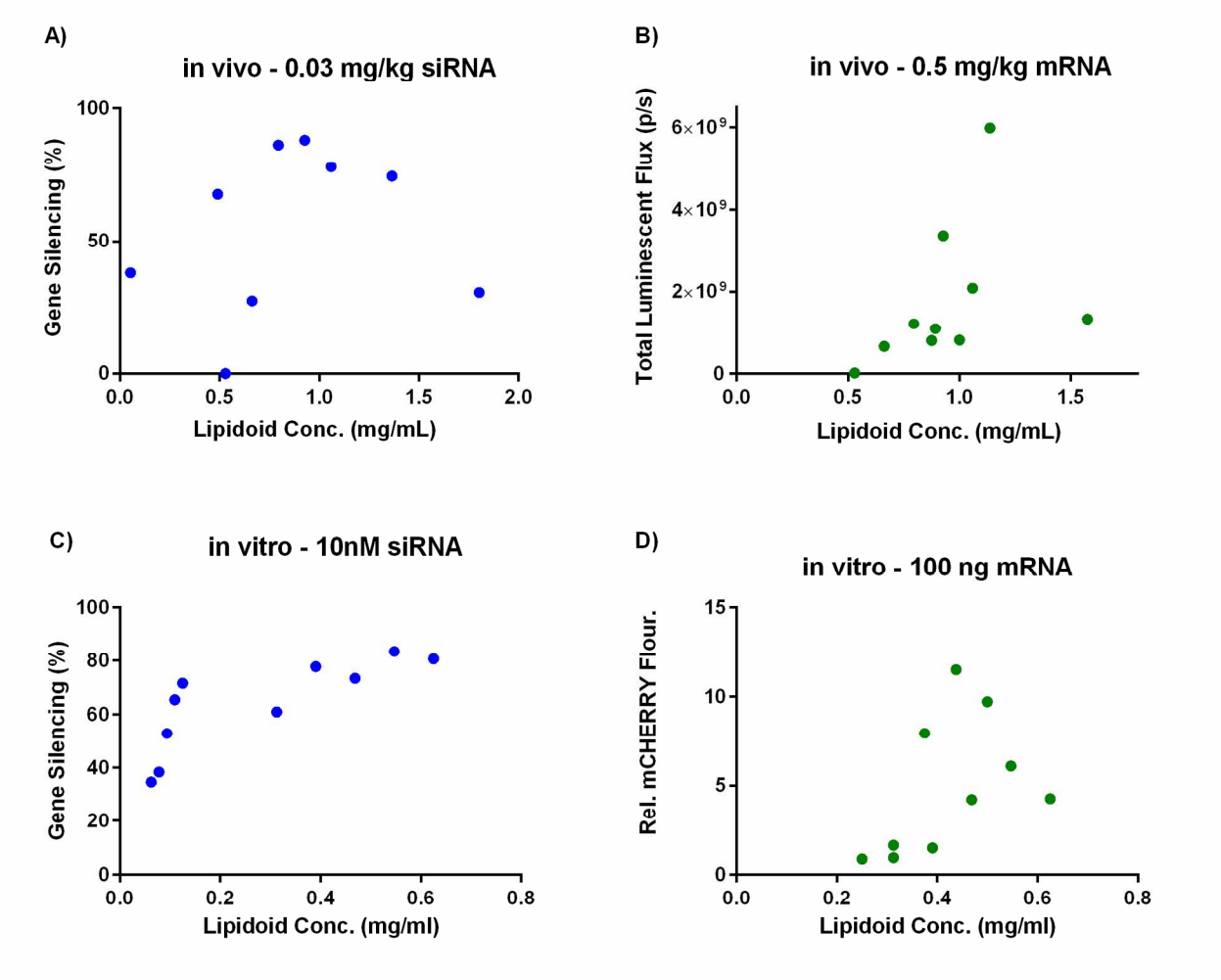

Figure S4. Neither **A)** siRNA-mediated Factor VII gene silencing nor **B)** mRNA-mediated luciferase expression in mice correlated with lipidoid concentration. All LNPs shown were dosed at 0.03 mg/kg siRNA and/or 0.5 mg/kg mLuc. **C, D**) A modest correlation between RNA delivery efficacy and lipidoid concentration was present in HeLa cells. The siRNA and mRNA doses were 10 nM and 100 ng, respectively. (n = 9 - 10).

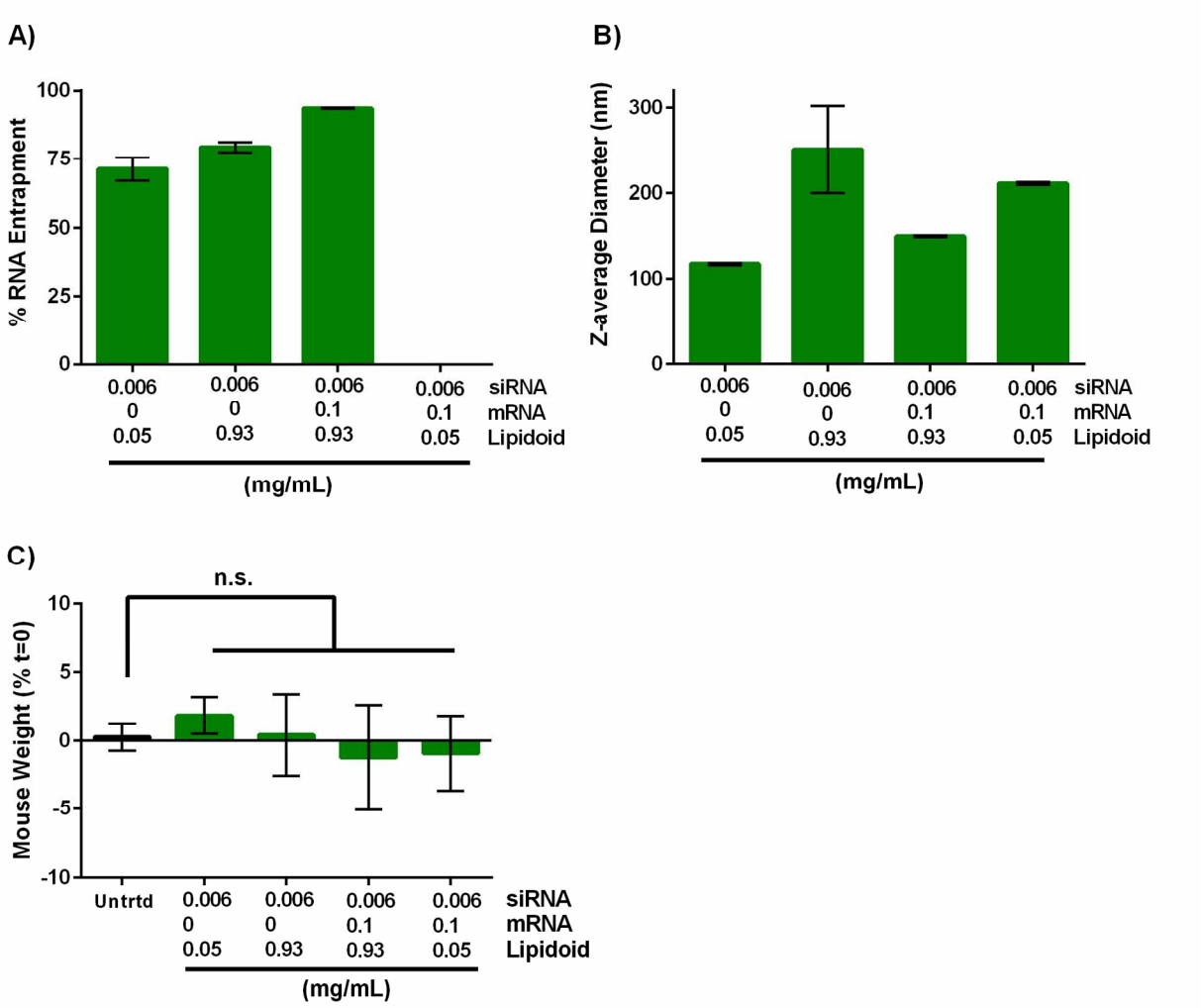

Figure S5. LNPs were formulated with siFVII alone, siFVII + mLuc (0.006 mg/mL) or LNPs with siFVII + mLuc (0.1 mg/mL) while keeping the lipidoid concentration constant. LNPs were delivered to mice at 0.03 mg/kg siFVII. **A)** LNPs did not form properly when formulated with 0.006 mg/mL siRNA, 0.1 mg/mL mLuc and a low concentration of lipidoid (0.05 mg/mL). (n = 3 technical replicates) **B)** The z-average diameter was measured using dynamic light scattering (n = 3 technical replicates). **C)** LNPs did not induce weight loss in mice compared to an untreated group. (n = 3) In all panels, error bars represent s.d.

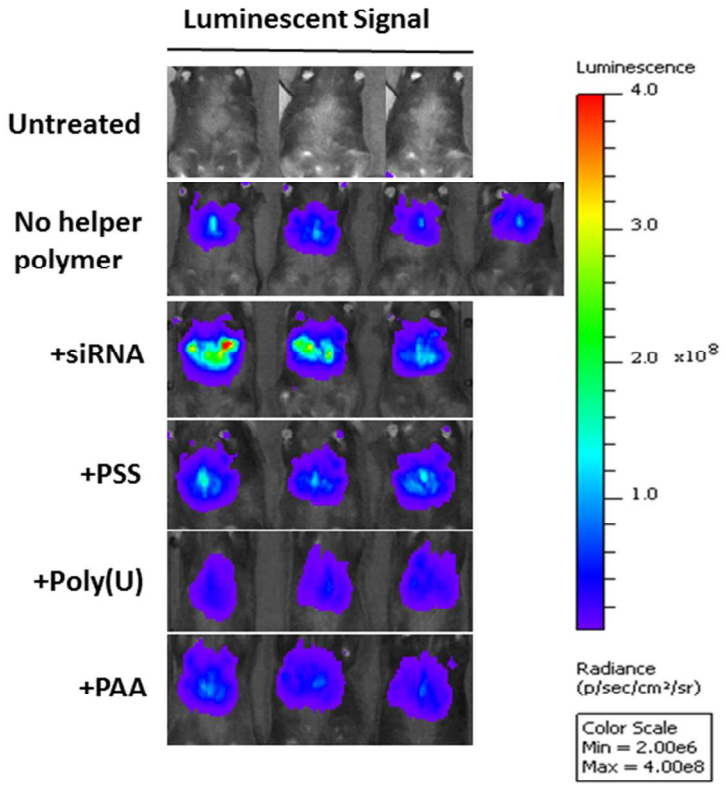

Figure S6: The addition of siFVII or PSS to the LNP formulation significantly enhanced mLuc delivery. LNPs were formulated using Formulation 4 with mLuc and either siFVII, PSS (6.8 kDa), Poly(U), or PAA. All LNPs were dosed at 0.5 mg/kg mLuc. Mice were imaged using an IVIS 6 hours post-LNP injection. (n = 3 - 4).

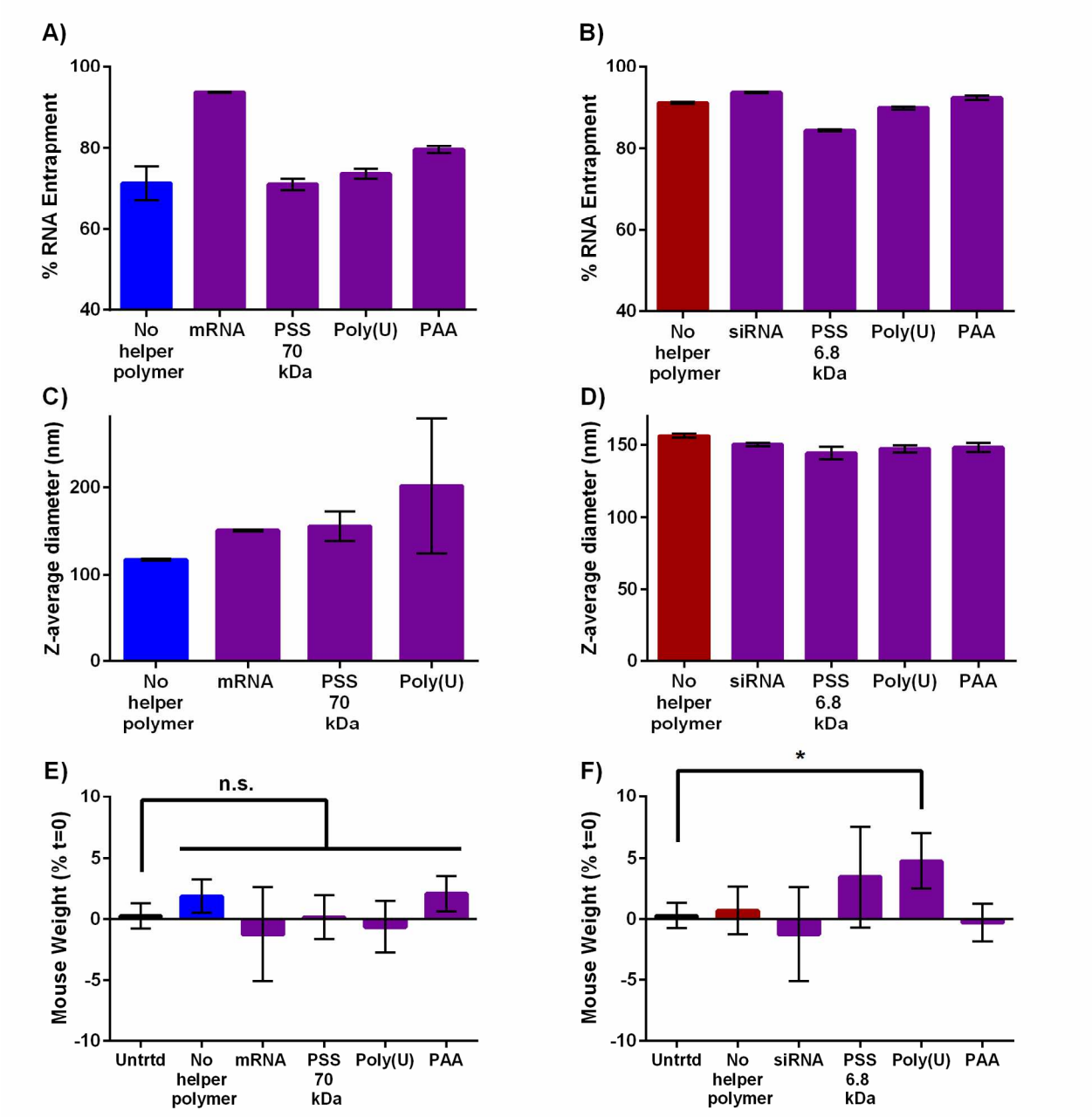

Figure S7. For panels **A, C, & E,** LNPs were formulated with siFVII and either mRNA and PSS (70 kDa), Poly(U), or PAA. For panels **B, D**, & **F,** LNPs were formulated with mLuc and either siRNA, PSS (6.8 kDa), Poly(U), or PAA. **A, B)** The addition of negatively charged polymers to the LNP formulation did not markedly increase total RNA entrapment (n = 3 technical replicates) **C, D)** LNP size ranged from 100 nm – 200 nm with or without the addition of negatively charged polymers in the formulation. (n = 3 technical replicates) **E, F)** LNPs did not result in weight loss compared to treated groups two days post-injection (n = 3). In all panels, error bars represent s.d.

LNPs co-formulated with siRNA and helper polymer					
Helper Polymer	**None**	**mRNA**	**PSS**	**Poly(U)**	**PAA**
Zeta Potential (mV)	-2.91 ± 0.54	-3.81 ± 0.65	-4.87 ± 0.77	-2.24 ± 0.62	-6.41 ± 3.85

Table S3. Zeta potential for the LNPs co-formulated with siRNA and a negatively charged helper polymer were not significantly different than LNPs formulated without helper polymer ("None"). These formulations correspond to those shown in Fig. 4A. Measured in PBS, pH 7.4. Values represent average ± standard deviation. (n = 3 technical replicates).

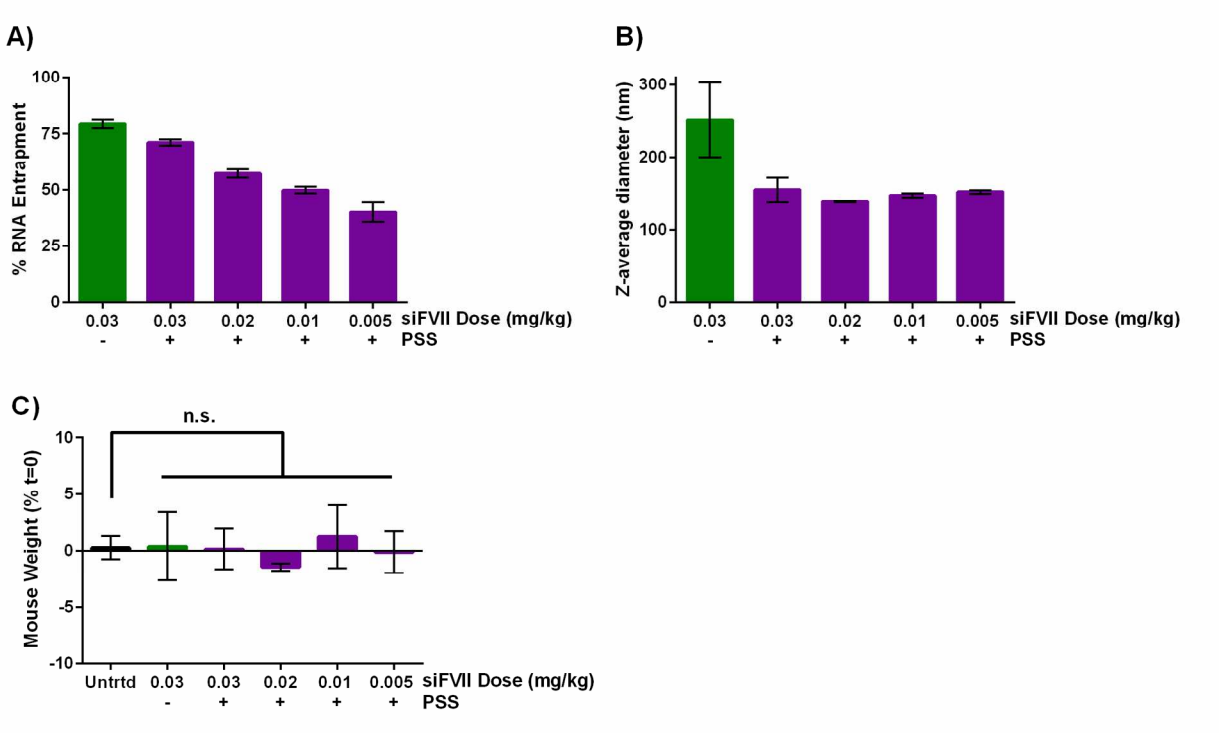

Figure S8. LNPs loaded with siFVII were formulated with or without PSS and delivered to mice at a range of siFVII doses (0.005 – 0.03 mg/kg). **A)** RNA entrapment decreased with decreasing siFVII dose. (n = 3 technical replicates) **B)** The z-average diameter was measured for each LNP using dynamic light scattering. (n = 3 technical replicates) **C)** Mice treated with LNPs did not experience weight change two days post-injection compared to untreated animals. (n = 3) In all panels, error bars represent s.d.