



Supplementary Figure S5: Impact of formulation conditions and resulting LNP size on FVII gene silencing in mice. Formulation of LNP contained DLin-KC2-DMA/ DSPC/ Cholesterol/ PEG-c-DMA at a molar ratio of 60/9.75/29.25/1. Sample LNP labeled ‘Optimal MF Mixing’ (●) were produced under fast mixing (4 ml/min) at a volumetric flow rate ratio of 3:1 (aq:EtOH) and were 60.0 nm in diameter. Lipid concentration was 35 mM in ethanol. Sample LNP labeled ‘Poor Mixing conditions’ (□) were produced at a 20 fold slower flow rate (0.2 ml/min) and at a higher lipid concentration (40 mM total lipid in ethanol). LNP were measured to be 170 nm in diameter. LNP siRNA-to-lipid ratio was maintained at 0.06 wt/wt. LNP diameter was determined by number-weighted mean diameter provided by DLS. Systemic injection of LNP-siRNA to mice was performed by tail vein injection (n=3 per dose level). Blood collection was performed after 24 hrs post-injection and factor VII levels were determined by colorimetric assay.