OMTN, Volume *29*

Supplemental information

PK-modifying anchors significantly alter clearance kinetics, tissue distribution, and efficacy of therapeutics siRNAs

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Supplementary table S1. List of oligonucleotides used in this study

See excel file.

Chemical modifications are designated as follow: '#', Phosphorothioate bond; 'Chol', Cholesterol; 'DCA', Docosanoic acid; 'DHA', Docosahexanoic acid; 'f', 2´-Fluoro; 'm', 2´-O-Methyl; 'P', 5´-Phosphate; 'Teg', Tetraethylene glycol; 'vP', 5´-Vinylphosphonate.

APOE, Apolipoprotein E; AS, Antisense strand; Gal, N-Acetylgalactosamine; HTT, Huntingtin; PEG, Polyethylene glycol; S, Sense strand; sFLT1, Soluble vascular endothelial growth factor receptor 1.

*These sequences were only partially complementary to the respective mRNA target. Nucleotides 1-15 or 1-17 of the guide strand were fully complementary to the target mRNA.

Supplementary table S2. Blood pharmacokinetic parameters of intravenously administered siRNA conjugates

Parameter	Unit	Gal	Gal PEG	DHA	DHA PEG	DCA	DCA PEG	Chol	Chol PEG
Cmax	μg/ml	54.8	333.4	241.5	396.3	165.0	306.1	178.6	248.3
AUC 0-24h	μg/ml*min	2053.4	42031.6	13040.9	113286.5	15958.3	72703.9	18956.8	94990.7
AUC 0-inf	μg/ml*min	2610.7	42199.4	13186.7	113496.5	16199.1	72829.3	19451.8	95205.4
Vz	(μg)/(μg/ml)	115.1	1.7	5.9	0.7	10.2	1.0	10.4	0.8
Cl	(μg)/(μg/ml)/min	0.13062	0.00808	0.02586	0.00300	0.02105	0.00468	0.01753	0.00358

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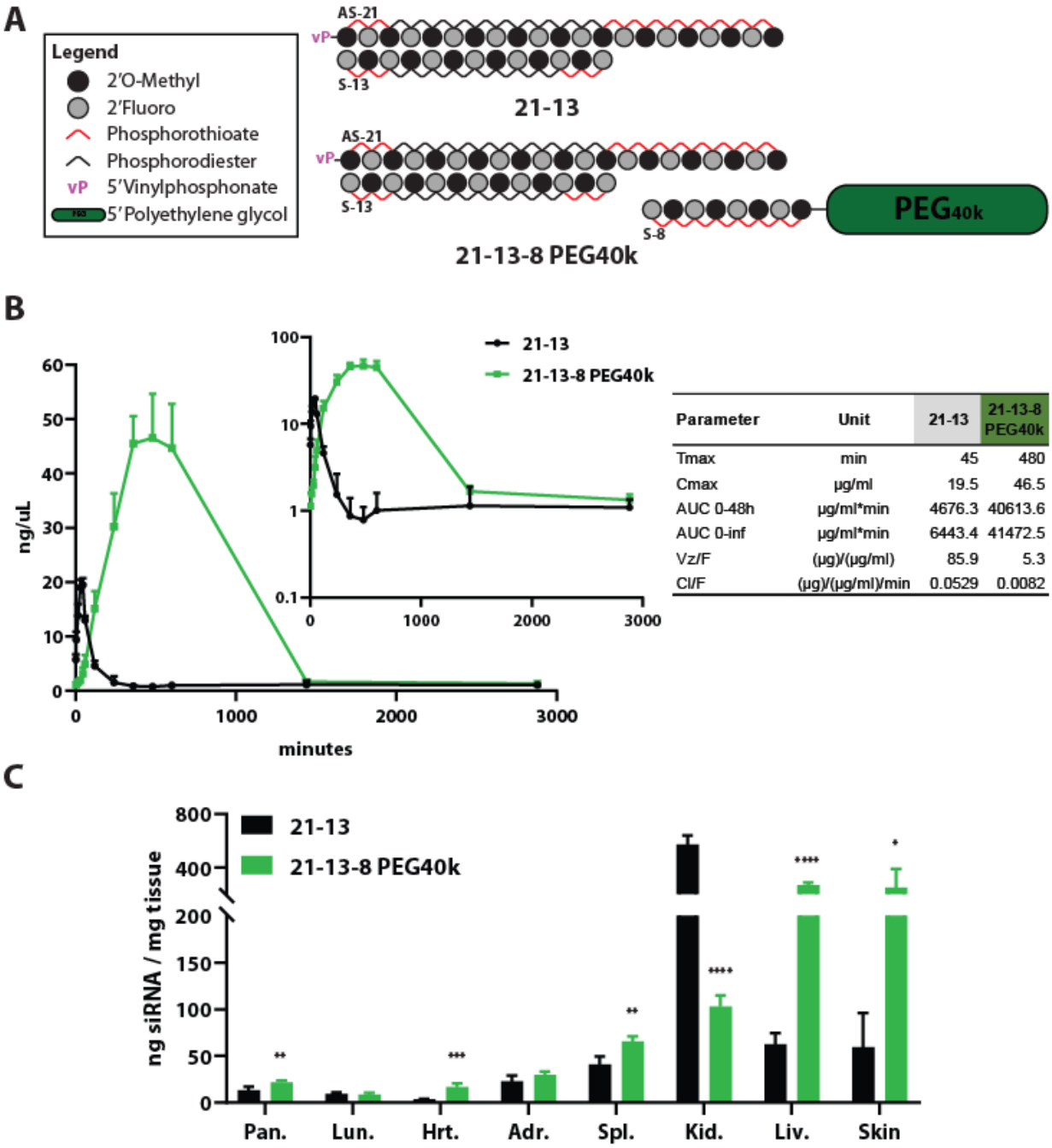
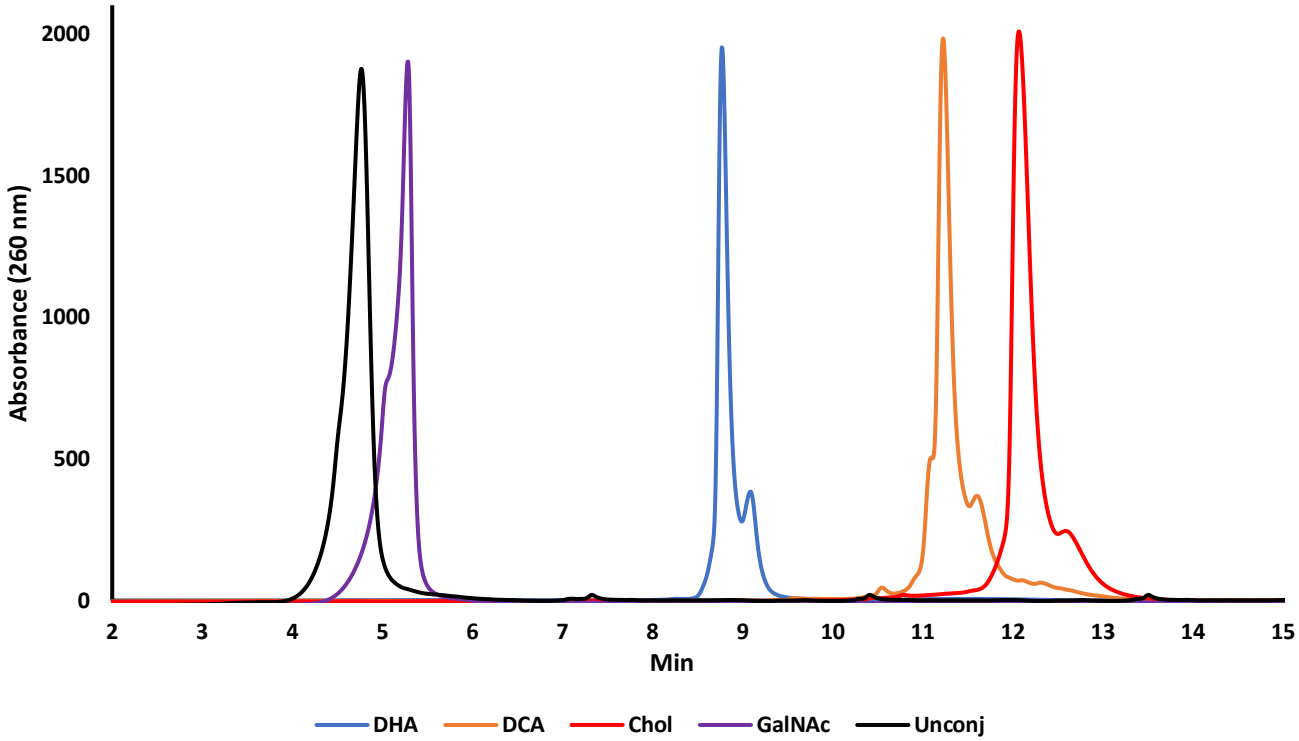
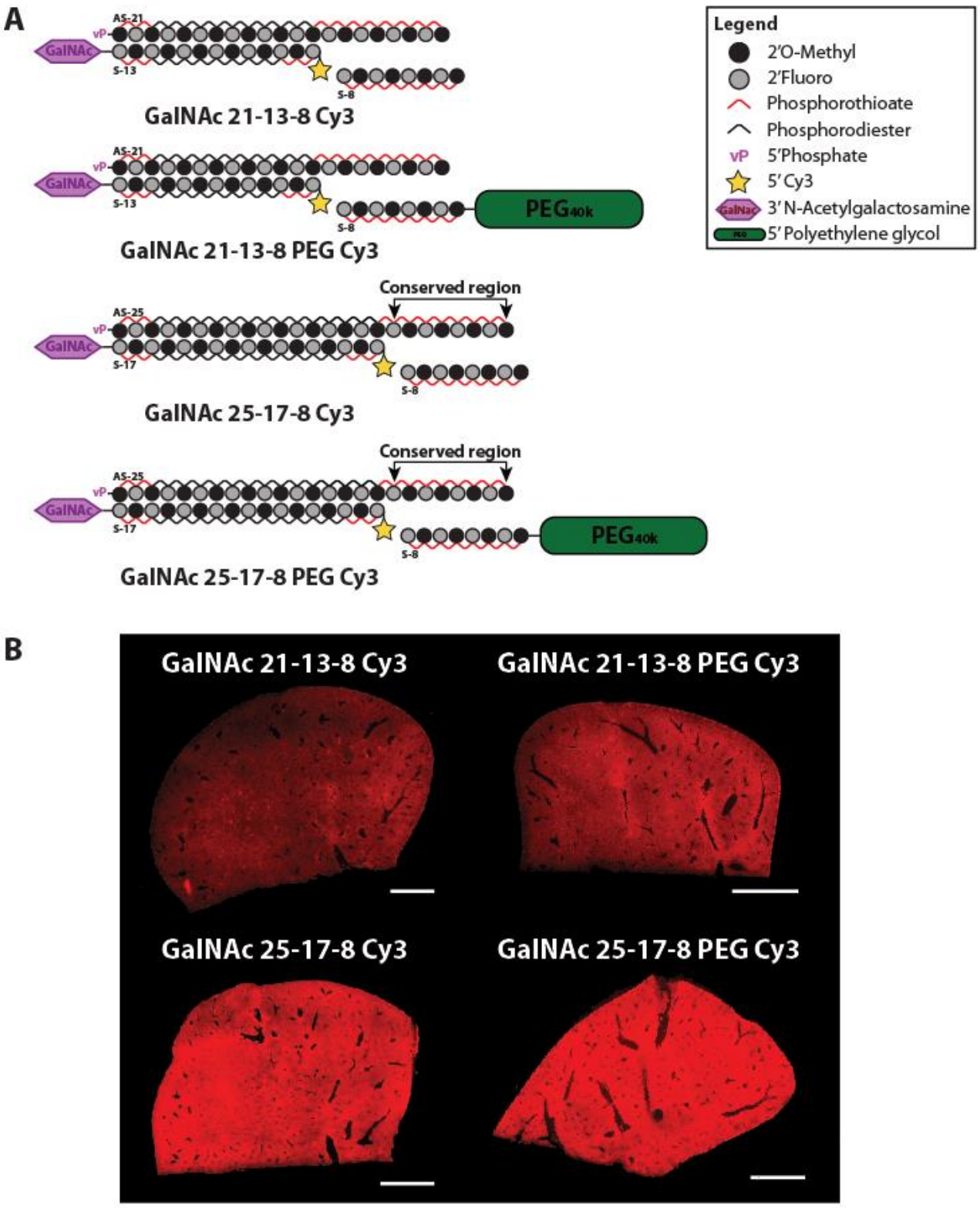
AUC, Area under the curve; Chol, Cholesterol; Cl, Clearance; Cmax, Maximum concetration; DCA, Docosanoic acid; DHA, Docosahexanoic acid; Gal, N-Acetylgalactosamine; PEG, Polyethylene glycol; t1/2α, distribution half-life; Vz, Volume of distribution.



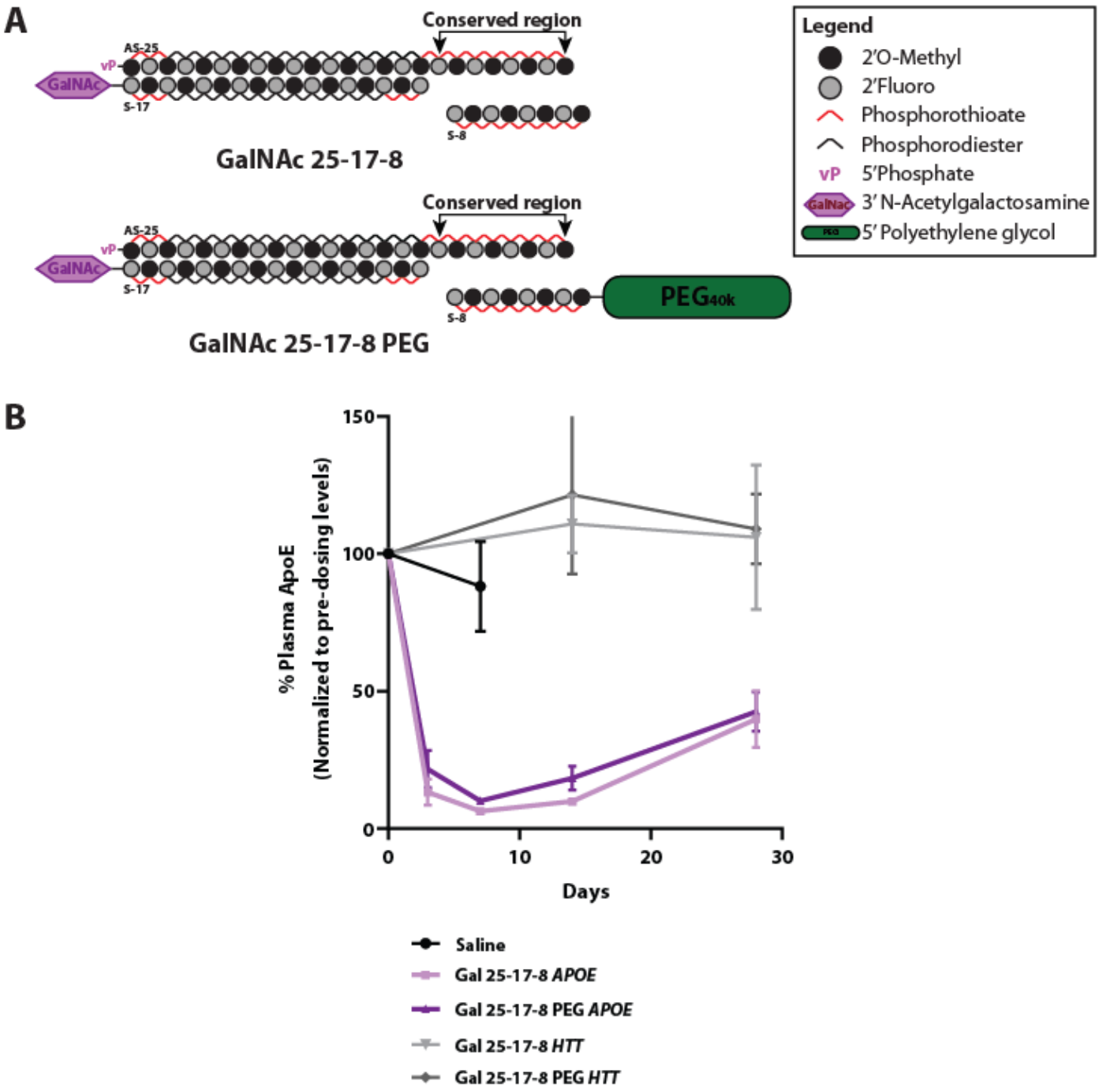
Supplementary Figure 1. PK-modifying anchors delay clearance and enhance tissue distribution of fully-modified asymmetric siRNAs after single subcutaneous administration. (A) (top) Schematic of fully modified asymmetric siRNA (21-13) and (bottom) schematic of an 8-mer oligonucleotide anchor conjugated to a 40 kDa polyethylene glycol (PEG) moiety binding to a parent asymmetric siRNA. (B, C) Wild-type FVB/N female mice treated subcutaneously (single dose, 28.5 nmol) with parent asymmetric siRNA duplex (21-13) or a PEGylated variant (21-13-8 PEG40k). Concentrations of the guide strand in the blood and tissues were assessed by PNA-based hybridization assay and values normalized to the MW of an unconjugated 21-13 asymmetric siRNA duplex. (B) Concentration-time profile for the parent asymmetric siRNA and corresponding PEGylated version using PK-modifying anchors. Serial blood samples were collected from the saphenous vein. (C) Tissue biodistribution profile assessed at 48 hours post-injection. n = 4-5/group.



Supplementary Figure 2. Conjugation of ligands to therapeutic oligonucleotides may significantly affect their physicochemical proprieties. High-performance liquid chromatography (HPLC) traces depicting differences in retention times of conjugated siRNA sense strands due to the inherent nature of the ligand. Reverse-phase HPLC performed using a C8 column.



Supplementary Figure 3. Standardized GC-rich PK-modifying anchors show modest improvement in delivery of parent GalNAc-conjugated siRNAs to the liver after single subcutaneous administration. (A) Schematics depict Cy3-labelled GalNAc-conjugated siRNA duplexes containing a GC-rich conserved region hybridizing to an 8-mer oligonucleotide anchor (with or without a polyethylene glycol (PEG) moiety). (B) Wild-type FVB/N female mice treated subcutaneously (single dose, 28.5 nmol) with Cy3-labelled GalNAc-conjugated siRNA duplexes as depicted above. Tiled fluorescent images of sections of the liver (10x objective. Scale bar, 2 mm) imaged at 48 hours post-injection. n = 3/group. Red: cy3-labelled oligonucleotide.



Supplementary Figure 4. GalNAc-conjugated siRNAs delivered with standardized GC-rich PK-modifying anchors enable potent downregulation of plasma APOE. (A) Schematics depict GalNAc-conjugated siRNA duplexes containing a GC-rich conserved region hybridizing to an 8-mer oligonucleotide anchor (with or without a polyethylene glycol (PEG) moiety). (B) Wild-type FVB/N female mice treated subcutaneously (single dose, 7.9 nmol (~5mg/kg of the parent asymmetric siRNA) with GalNAc-conjugated siRNA duplexes as depicted above. Blood samples were collected from mandibular bleeds at pre-dosing and 3-, 7-, 14- and 28-days post-injection. Serum ApoE was quantified by ELISA and data displayed as percent change from pre-dosing levels. n = 5/group.