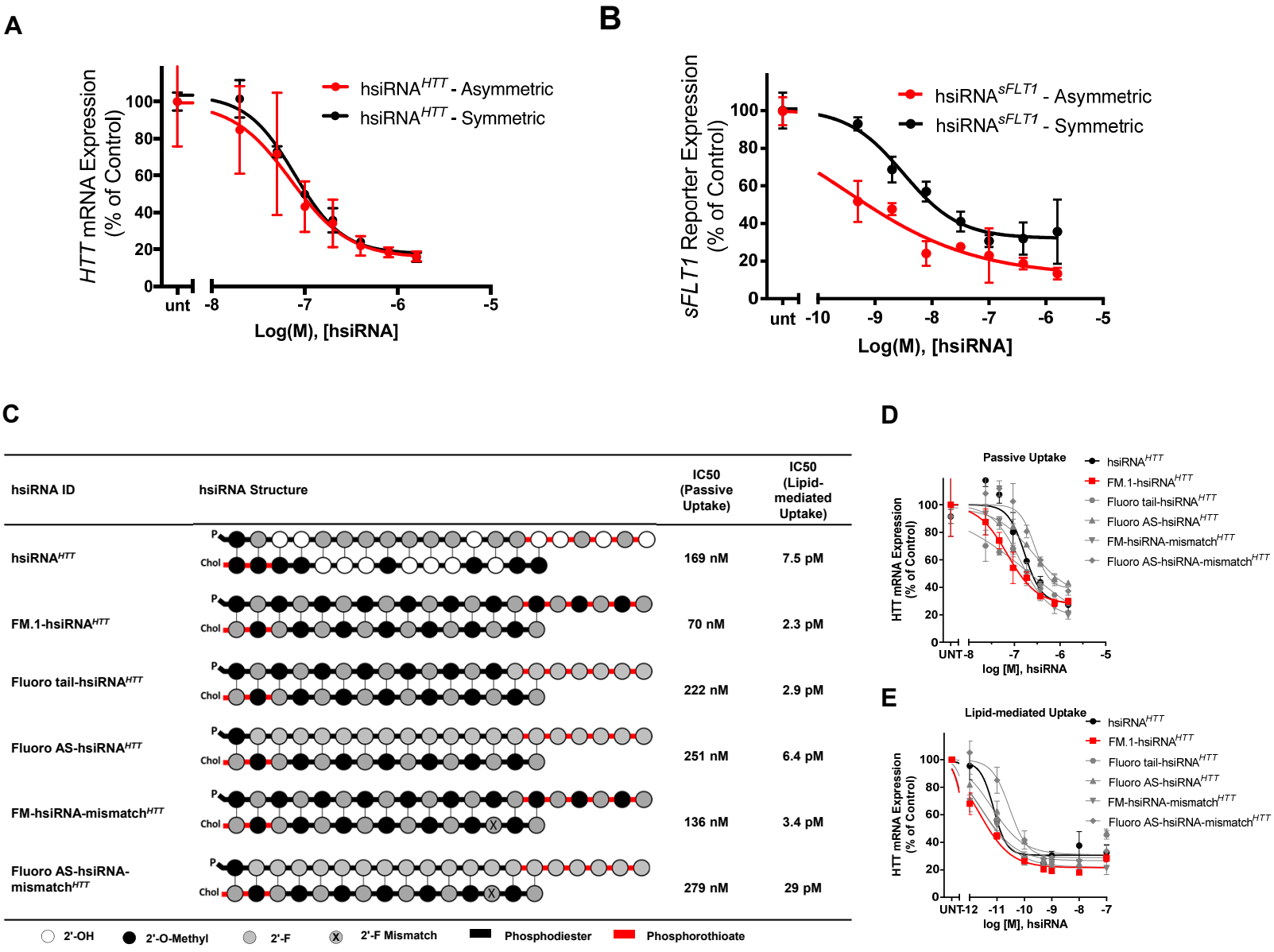
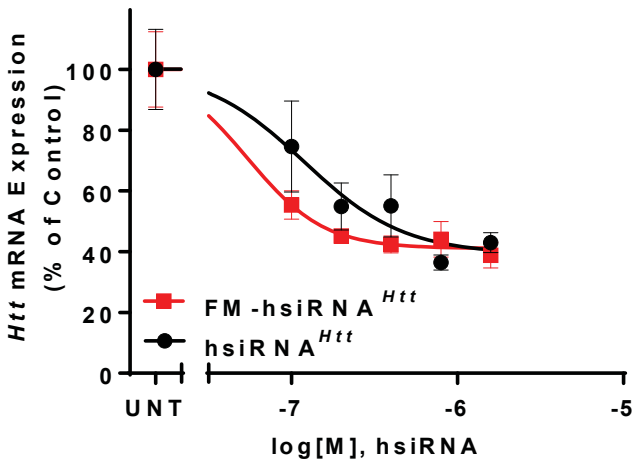
Supplementary Figure 1



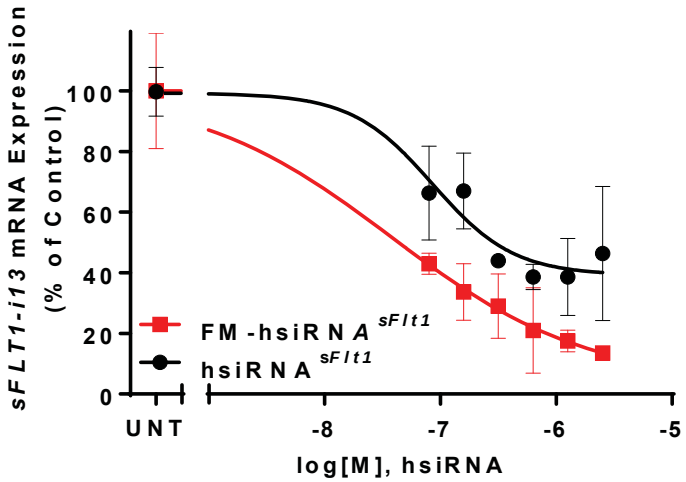
Supplamental Figure 1. A comparison of symmetric and asymmetric siRNAs in *vitro* and screen of alternative FM-hsiRNA^{HTT} patterns. (**A,B**) The effect of duplex chemical configuration on siRNA activity is sequence dependent . HeLa cells were treated in the absence of cationic lipids with asymmetric and symmetric siRNAs targeting both *HTT* and *sFLT1*. *HTT* expression was measured using QuantiGene 2.0 (Affymetrix), normalized to the housekeeping gene *HPRT* and *sFLT1* expression was measured using a luciferase reporter assay (**C**) Representative images of alternative modification patterns and their corresponding IC50s. IC50s calculated as described in Materials and Methods. Cells treated for 72 hours with compound in the (**D**) absence (**E**) or presence of cationic lipids. *HTT* mRNA was measured using QuantiGene 2.0 (Affymetrix), normalized to the housekeeping gene *PPIB*. All data is expressed as percent of untreated control . (n=3 wells, mean ± SD). UNT – untreated cells.

Supplementary Figure 2

A



B

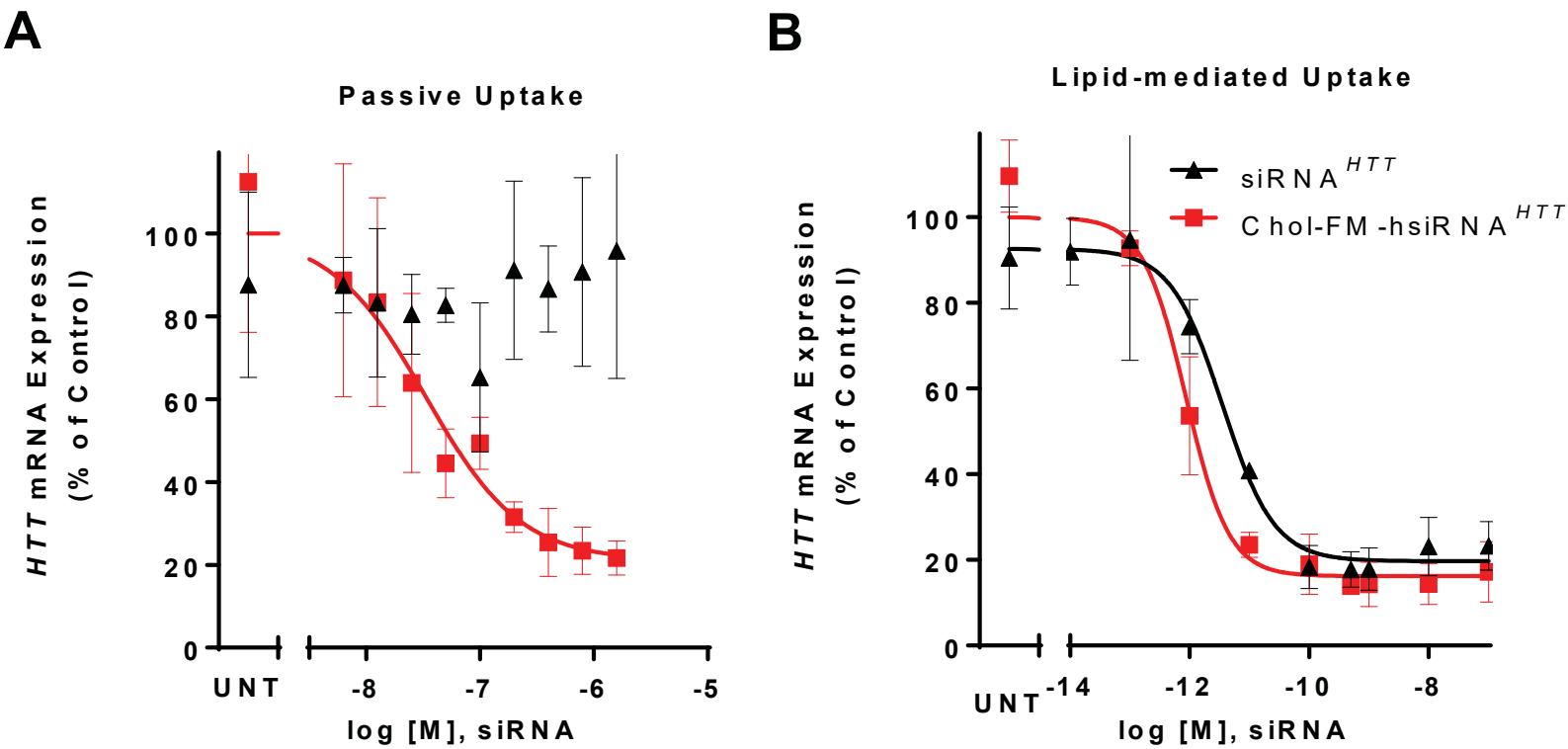


C

IC50 (Passive Uptake)	hsiRNA	FM-hsiRNA
Primary neurons (targeting *Htt*)	119 nM	54.9 nM
Primary trophoblasts (targeting *sFlt1*)	85.7 nM	37.5 nM

Supplementary Figure 2. Comparison of hsiRNA and FM-hsiRNA activity in *vitro*. Primary neurons (**A**) or primary cytotrophoblasts (**B**) were incubated with hsiRNA or FM-hsiRNA at concentrations shown for one week. mRNA levels were measured using QuantiGene (Affymetrix) normalized to housekeeping gene (mouse *Ppib* for (**A**) and human *Ywhaz* for (**B**)), and presented as percent of untreated control (n=3, mean ± SD). UNT – untreated cells. IC50 values were calculated as described in Materials and Methods and are presented in table (**C**).

Supplementary Figure 3

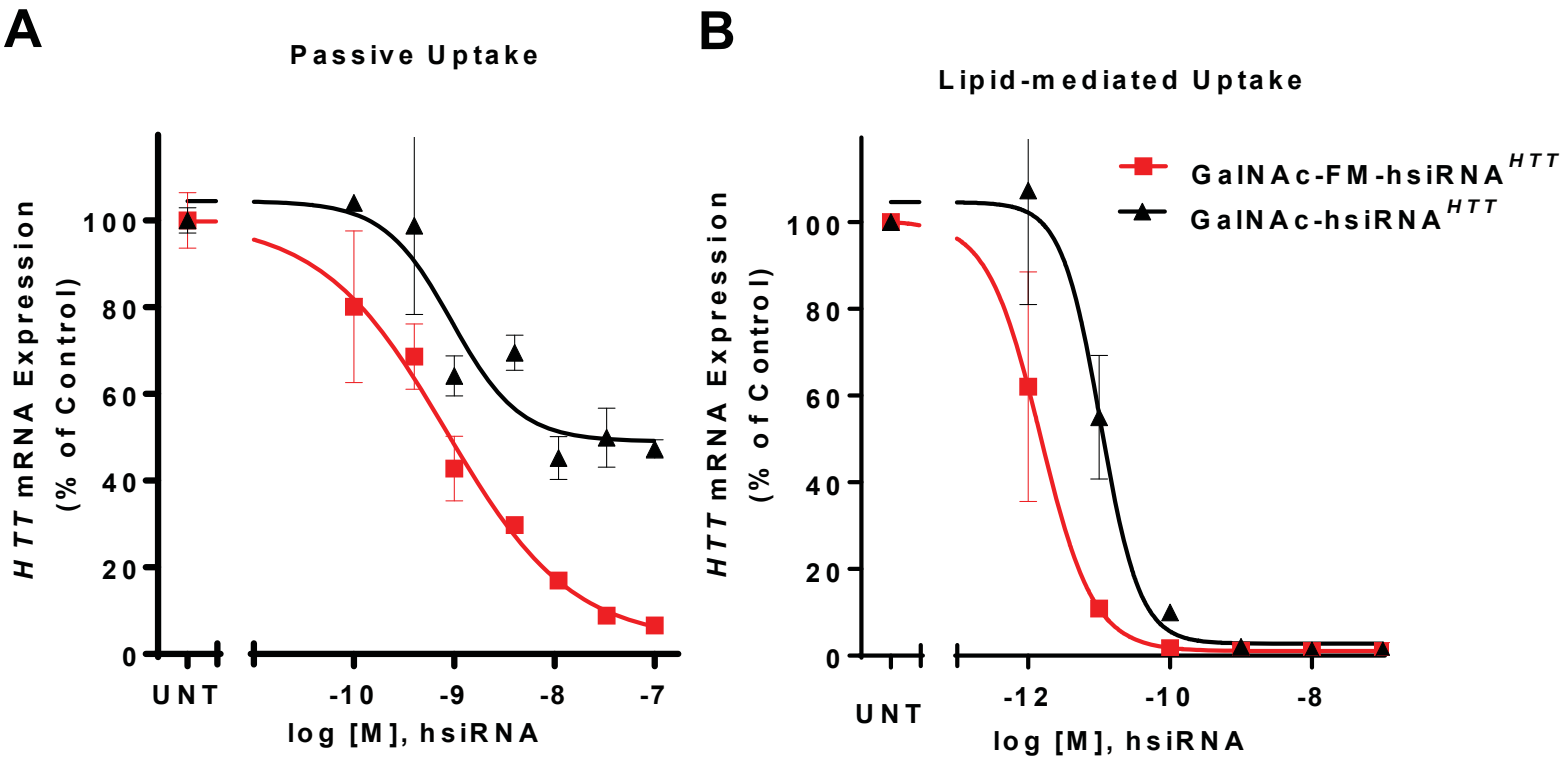


B

	siRNA^{HTT}	**Chol-FM-hsiRNA^{HTT}**
IC50 (Passive Uptake)	N/A	33.5 nM
IC50 (Lipid Mediated Uptake)	3.5 pM	0.9 pM

Supplementary Figure 3. Full chemical modification is essential for unassisted cellular delivery and does not compromise siRNA RISC entry. HeLa Cells treated for 72 hours with compound in the (**A**) absence or (**B**) presence of cationic lipids. *HTT* mRNA was measured using QuantiGene®, normalized to the housekeeping gene *PPIB*, and expressed as percent of untreated control. (n=3 wells, mean ± SD). UNT – untreated cells. IC50 values were calculated as described in Materials and Methods and are presented in table (**C**).

Supplementary Figure 4

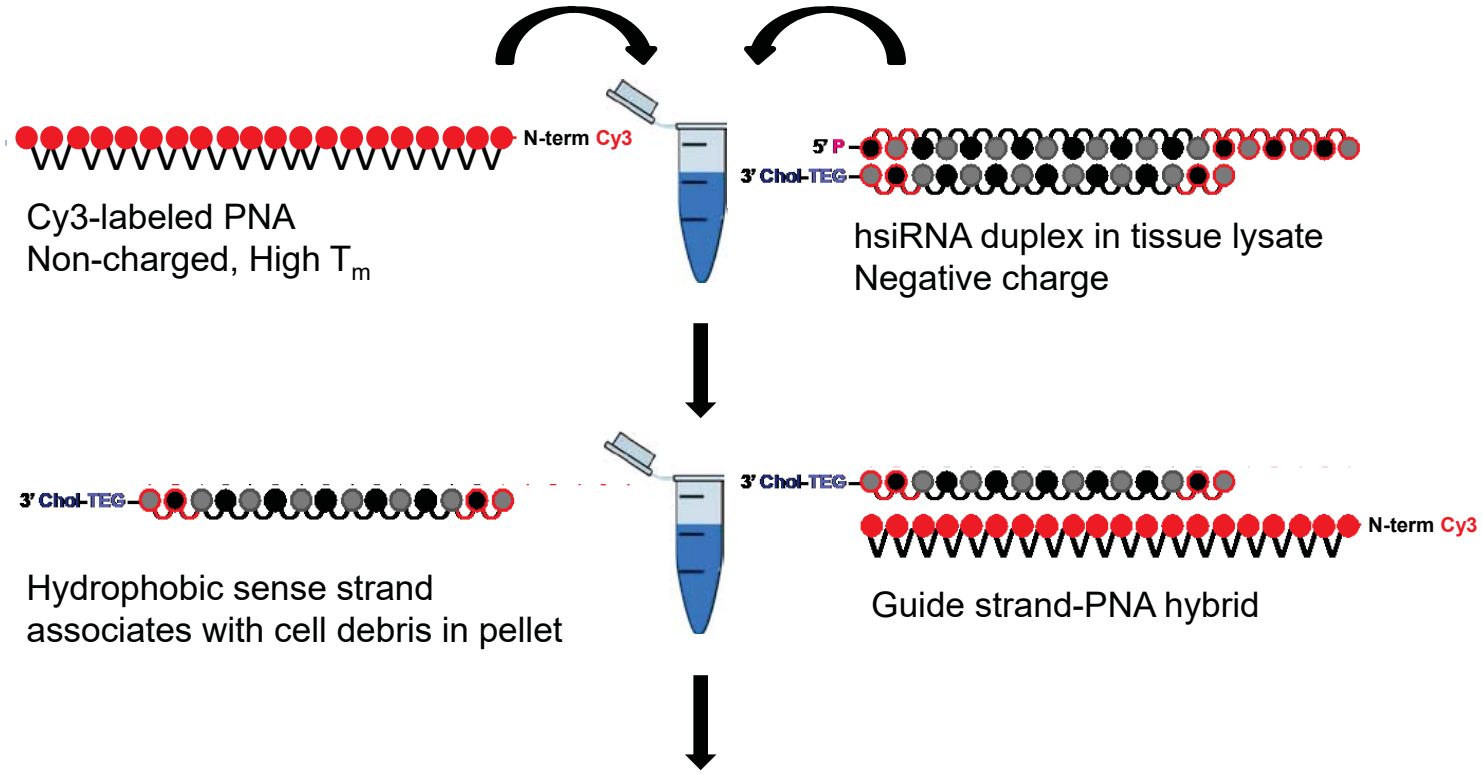


C

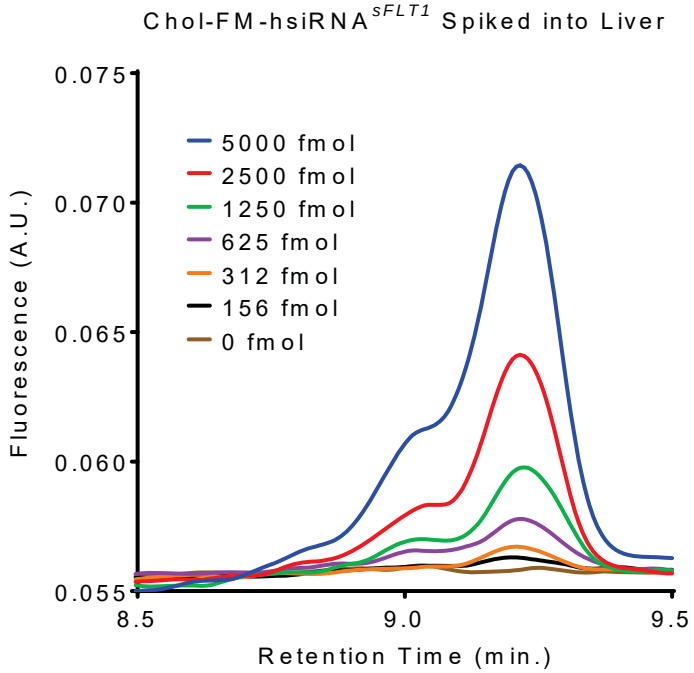
	GalNAc-hsiRNA^{HTT}	GalNAc-FM-hsiRNA^{HTT}
IC50 (Passive Uptake)	940 pM	830 pM
IC50 (Lipid Mediated Uptake)	10 pM	1.5 pM

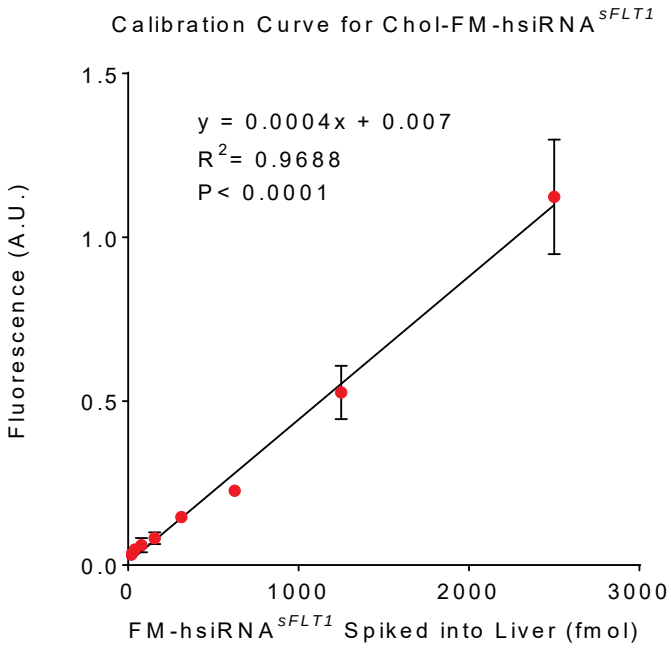
Supplementary Figure 4 Full chemical modification enhances GalNAc-mediated mRNA silencing in human primary hepatocytes. Cells treated for 72 hours with compound in the (**A**) absence or (**B**) presence of cationic lipids. HTT mRNA was measured using QuantiGene®, normalized to the housekeeping gene PPIB, and expressed as percent of untreated control. (n=3 wells, mean ± SD). UNT – untreated cells. IC50 values were calculated as described in Materials and Methods and are presented in table (**C**).

Supplementary Figure 5



Anion Exchange HPLC Separation (DNAPac PA100)





Supplementary Figure 5 PNA (Peptide Nucleic Acid) hybridization-based assay for detection of hsiRNAs in mouse tissues. A Cy3-labeled PNA fully complementary to the guide strand out-competes the passenger strand and/or any mRNA targets the guide strand might be bound to. Then, the guide strand – PNA hybrid is separated from other components of tissue lysates by anion exchange chromatography. A calibration curve was derived by spiking in known amount of hsiRNA into tissue lysates.

Supplementary Table 1

Table 1 Chemical modification patterns and sequences of hsiRNAs

siRNA ID	Gene	Accession number	Targeting Position	Sense	Antisense	Conjugate
Chol-hsiRNA^{HTT}	HTT	NM_002111.6	10150	mC.mA.G.mU.A.A.A.mG.A.G.A.mU.mU#mA#mA	PmU.fU.A.A.fU.fC.fU.fC.fU.fU.fU.A.fC.fU#G#A#fU#A#fU#A	Teg-cholesterol
Chol-FM.1-hsiRNA^{HTT}	HTT	NM_002111.6	10150	fC.mA.fG.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA#	PmU.fU.mA.fA.mU.fC.mU.fC.mU.fU.mU.fA.mC.fU#mG#fA#mU#fA#mU#fA	Teg-cholesterol
Chol-Fluoro tail-hsiRNA^{HTT}	HTT	NM_002111.6	10150	fC.mA.fG.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA#	PmU.fU.mA.fA.mU.fC.mU.fC.mU.fU.mU.fA.mC.fU#fG#fA#fU#fA#fU#fA	Teg-cholesterol
Chol-Fluoro AS-hsiRNA^{HTT}	HTT	NM_002111.6	10150	fC.mA.fG.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA#	PmU.fU.fA.fA.fU.fC.fU.fC.fU.fU.fU.fA.fC.fU#fG#fA#fU#fA#fU#fA	Teg-cholesterol
Chol-FM.1-mismatch-hsiRNA^{HTT}	HTT	NM_002111.6	10150	fC.mA.fU.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA#	PmU.fU.mA.fA.mU.fC.mU.fC.mU.fU.mU.fA.mC.fU#mG#fA#mU#fA#mU#fA	Teg-cholesterol
Chol-Fluoro AS-mismatch-hsiRNA^{HTT}	HTT	NM_002111.6	10150	fC.mA.fU.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA#	PmU.fU.fA.fA.fU.fC.fU.fC.fU.fU.fU.fA.fC.fU#fG#fA#fU#fA#fU#fA	Teg-cholesterol
Chol-FM-hsiRNA^{HTT}	HTT	NM_002111.6	10150	fC#mA#fG.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA	PmU#fU#mA.fA.mU.fC.mU.fC.mU.fU.mU.fA.mC#fU#mG#fA#mU#fA#mU#fA	Teg-cholesterol
Chol-hsiRNA^{sFLT1}	sFLT1	NM_001159920	2283	mG.mA.mU.mC.mU.mC.mC.A.A.A.mU.mU.mU#mA#mA	PmU.A.A.A.fU.fU.fU.G.G.mA.G.A.fU.fC#fC#G#A#G#A#G	Teg-cholesterol
Chol-FM-hsiRNA^{sFLT1}	sFLT1	NM_001159920	2283	fG#mG#fA.mU.fC.mU.fC.mC.fA.mA.fA.mU.fU#mU#fA	PmU#fA#mA.fA.mU.fU.mU.fG.mG.fA.mG.fA.mU#fC#mC#fG#mA#fG#mA#fG	Teg-cholesterol

Chol-hsiRNA^{PPIB}	PPIB	NM_009693.2	437	mC.mA.A.A.mU.mU.mC.mC.A.mU.mC.G.mU#mG#mA#	PmU.fC.A.fC.G.A.fU.G.G.mA.A.fU.fU.fU#G#fC#fU#G#U#U	Teg-cholesterol
Chol-FM-hsiRNA^{PPIB}	PPIB	NM_009693.2	437	fC#mA#fA.mA.fU.mU.fC.mC.fA.mU.fC.mG.fU#mG#fA	PmU#fC#mA.fC.mG.fA.mU.fG.mG.fA.mA.fU.mU#fU#mG#fC#mU#fG#mU#fU	Teg-cholesterol
GalNAc-hsiRNA^{HTT}	HTT	NM_002111.6	10150	mC.mA.G.mU.A.A.A.mG.A.G.A.mU.mU#mA#mA	PmU.fU.A.A.fU.fC.fU.fC.fU.fU.fU.A.fC.fU#G#A#fU#A#fU#A	GalNAc
GalNAc-FM-hsiRNA^{HTT}	HTT	NM_002111.6	10150	fC#mA#fG.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA	PmU#fU#mA.fA.mU.fC.mU.fC.mU.fU.mU.fA.mC#fU#mG#fA#mU#fA#mU#fA	GalNAc
siRNA^{HTT}	HTT	NM_002111.6	10150	mA.mU.A.U.C.A.G.U.A.A.A.G.A.G.A.U.U.A.A.U.U	PU.U.A.A.U.C.U.C.U.U.U.A.C.U.G.A.U.A.U.U.U	none
DHA-hsiRNA^{sFLT1}	sFLT1	NM_001159920	2283	mG.mA.mU.mC.mU.mC.mC.A.A.A.mU.mU.mU#mA#mA	PmU.A.A.A.fU.fU.fU.G.G.mA.G.A.fU.fC#fC#G#A#G#A#G	DHA
DHA-FM-hsiRNA^{sFLT1}	sFLT1	NM_001159920	2283	fG#mG#fA.mU.fC.mU.fC.mC.fA.mA.fA.mU.fU#mU#fA	PmU#fA#mA.fA.mU.fU.mU.fG.mG.fA.mG.fA.mU.fC#mC#fG#mA#fG#mA#fG	DHA
DHA-FM-hsiRNA^{PPIB}	PPIB	NM_009693.2	437	fC#mA#fA.mA.fU.mU.fC.mC.fA.mU.fC.mG.fU#mG#fA	PmU#fC#mA.fC.mG.fA.mU.fG.mG.fA.mA.fU.mU#fU#mG#fC#mU#fG#mU#fU	DHA
^{V}Chol-hsiRNA^{PPIB}	PPIB	NM_009693.2	437	mC.mA.A.A.mU.mU.mC.mC.A.mU.mC.G.mU#mG#mA#	VmU.fC.A.fC.G.A.fU.G.G.mA.A.fU.fU.fU#G#fC#fU#G#U#U	Teg-cholesterol
^{V}Chol-FM-hsiRNA^{PPIB}	PPIB	NM_009693.2	437	fC#mA#fA.mA.fU.mU.fC.mC.fA.mU.fC.mG.fU#mG#fA	VmU#fC#mA.fC.mG.fA.mU.fG.mG.fA.mA.fU.mU#fU#mG#fC#mU#fG#mU#fU	Teg-cholesterol
^{V}Chol-hsiRNA^{HTT}	HTT	NM_002111.6	10150	mC.mA.G.mU.A.A.A.mG.A.G.A.mU.mU#mA#mA	VmU.fU.A.A.fU.fC.fU.fC.fU.fU.fU.A.fC.fU#G#A#fU#A#fU#A	Teg-cholesterol

^{V}Chol-FM-hsiRNA^{HTT}	HTT	NM_002111.6	10150	fC#mA#fG.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA	VmU#fU#mA.fA.mU.fC.mU.fC.mU.fU.mU.fA.mC#fU#mG#fA#mU#fA#mU#fA	Teg-cholesterol
^{V}Chol-hsiRNA^{sFLT1}	sFLT1	NM_001159920	2283	mG.mA.mU.mC.mU.mC.mC.A.A.A.mU.mU.mU#mA#mA	VmU.A.A.A.fU.fU.fU.G.G.mA.G.A.fU.fC#fC#G#A#G#A#G	Teg-cholesterol
^{V}Chol-FM-hsiRNA^{sFLT1}	sFLT1	NM_001159920	2283	fG#mG#fA.mU.fC.mU.fC.mC.fA.mA.fA.mU.fU#mU#fA	VmU#fA#mA.fA.mU.fU.mU.fG.mG.fA.mG.fA.mU#fC#mC#fG#mA#fG#mA#fG	Teg-cholesterol
PNA^{sFLT1}	sFLT1	NM_001159920	2283	C*T*C*T*C*G*G*A*T*C*T*C*C*A*A*A*T*T*T*A		CY3 -(OO)-

Chemical modifications are designated as follows. "." – phosphodiester bond, "#" – phosphorothioate bond, "m" – 2'-*O*-Methyl, "f" – 2'-Fluoro, no prefix – ribonucleotide, "P" – 5' Phosphate, , "V" – 5'-(E)-Vinyl phosphonate, "teg-cholesterol" – tetraethylene glycol (teg)-Cholesterol, GalNAc – trivalent N-Acetylgalactosamine, (OO) – O-linker (PNA Bio), DHA – docosahexaenoic acid.