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A. Validation of Extraction and HPLC Method for Measuring siRNA in Brain Tissue

We developed a novel extraction protocol allowing full recovery of siRNA to perform measurements of its distribution across the brain delivered via the inranasal instillation of the nanoparticles carried Cy-3 labeled siRNA, The major part of this protocol was to develop a special Extraction Buffer suitable for HPLC analysis with anion-exchange DNA Pac PA100 column. This column cannot be used with anionic detergents as they bind irreversible to the column. The known extraction method described in US2011020100 Patent traditionally uses anionic detergent SDS for extraction of siRNA but requires its removal before injection to the column by using high concentration of KCl. Testing of this method in our lab showed that KCL co-precipitates siRNA together with SDS (Fig S1). The loss of siRNA reaches 95% making such approach unfeasible for quantitative determination of siRNA delivery to the brain.

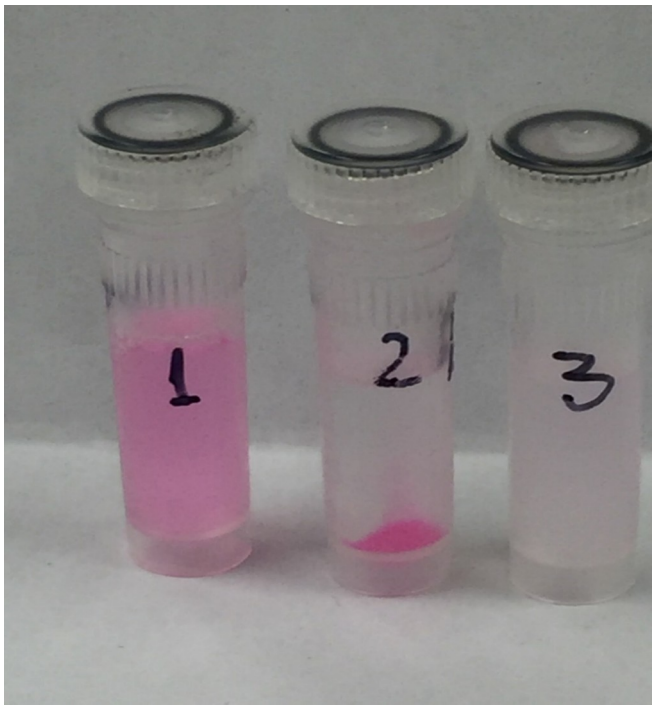

Fig S1. Effect of precipitation of hsiRNA by KCl according to standard protocol (patent US2011020100).

1. hsiRNA dissolved in 1 mL of Extraction Buffer buffer at concentration 1.5 uM;
2. 200 uL of 3M KCl added to hsiRNA solution;
3. Supernatant is separated from pellet after centrifugationat 10 000 g for 2 min.

New Extraction Buffer contains the following components:

25 mM Tris-HCl, pH 7.4
20mM NaCl
1mM EDTA

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0.5% EMPIGEN

Utilization of zwitterionic detergent EMPIGEN in recipe allowed to recover not less than 96% of siRNA from the brain tissue.

The protocol of extraction has included the following procedures.

a. Homogenization of brain tissue with Tissue Raptor (Quiagen) in prepared Extraction Buffer with proportion of 10 to 1 (v/wt); for example, 10 uL/mg
b. Centrifugation samples @10,000 rpm x 15 min
c. Transfer supernatant to new tubes
d. Adding 2 mg/mL of Proteinase K (EpiCentre, Cat# MPRK092) to lyse proteins
e. Lysis of proteins @ 50 C for 20 min
f. Centrifugation samples @10,000 rpm x 15 min
g. Transfer supernatant to Costar Spin-X Centrifuge tube filters, 0.45 µm (Cole-Parmer, Cat # UX-01937-40)
h. Centrifuge @ 14,000 rpm x 10 min
i. Transfer filtered samples into HPLC vials

Analysis was performed on Perking Elmer series 200 HPLC with auto sampler and both UV and Fluorescent detector. Binary gradient pump delivers two eluents A and B programmed to crate gradient suitable for elution of siRNA.
Eluent A: 50 mM Tris Buffer, 2 mM EDTA and 50% acetonitrile (pH 8.0)
Eluent B: 50 mM Tris Buffer, 2 mM EDTA; 2.6 M NaClO4 and 50% acetonitrile (pH 8.0)
The pump was programmed for creation of gradient as depicted on Fig. S2.

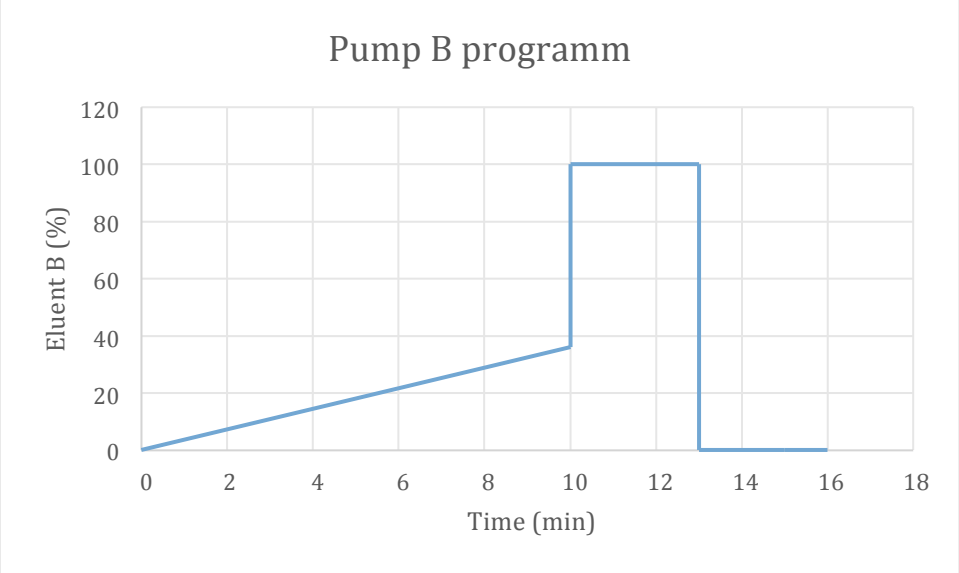

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Fig. S2 Programmed gradient of eluent B.

The typical chromatogram representing siRNA pick with retention time 6.7 min at flow 1 ml/min is depicted by Fig. 3.

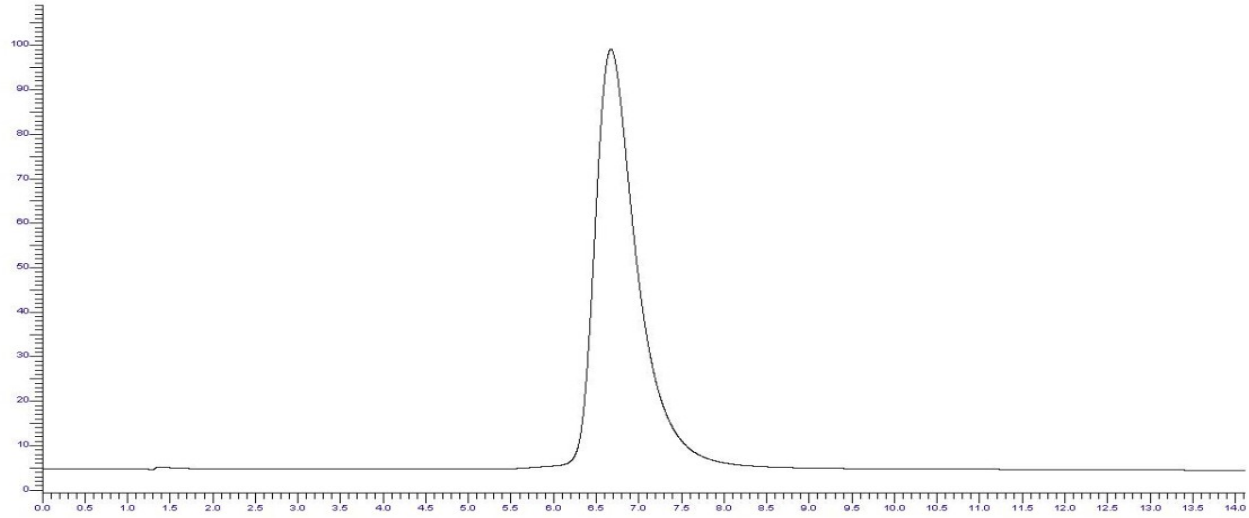

Fig. S3 HPLC chromatogram of 6.1 pmol of siRNA

Calibration curve obtained with different concentrations of siRNA depicted by Fig. S4.

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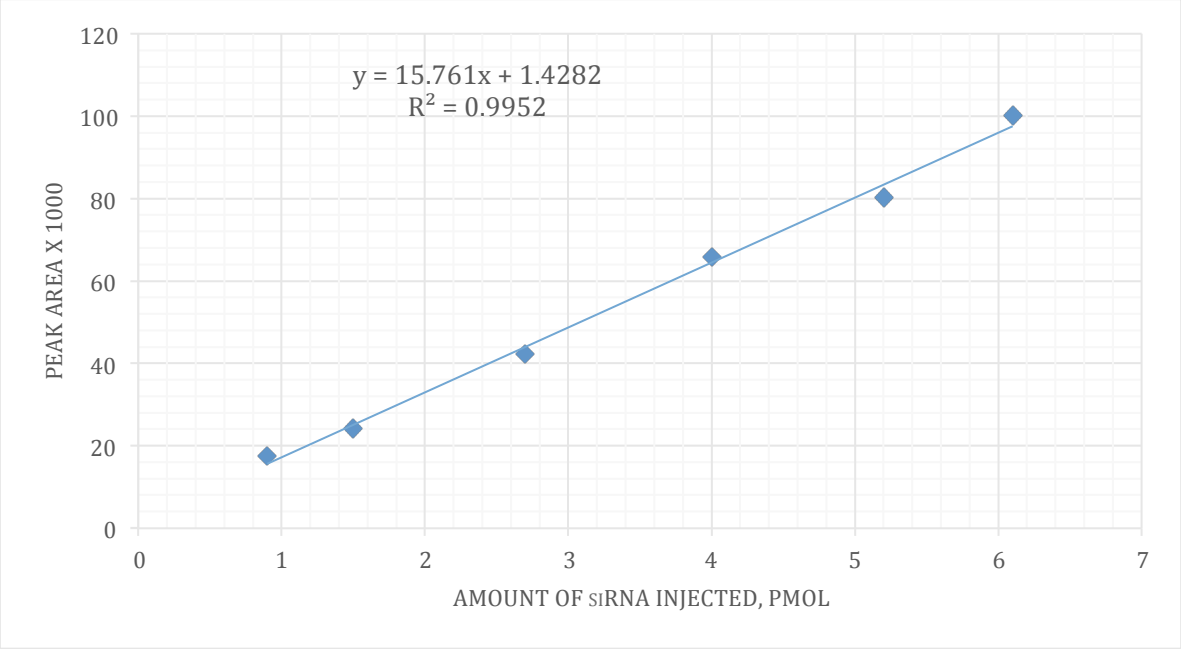

Fig.S4 Calibration curve for quantitative HPLC determination of siRNA extracted from mouse brain.

Validation of HPLC method for quantitative determination of Cy-3siRNA extracted from mouse brain revealed the following parameters (Table S1):

Table S1	
Recovery Mean	101.09%
Recovery SD	6.71%
Limit of Detection	1.09 pmol
Limit of Quantitation	3.31 pmol
Dynamic Range	0.9 – 6.1 pmol

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B Detailed Method for Synthesis of hsiRNA

Hydrophobically modified siRNA (hsiRNA) against htt (HTT10150) hsiRNA was based on a previously identified HTT functional targeting site(13).The compounds were asymmetric, composed of a 15-nucleotide long duplex region with a single-stranded 3′ extension on the guide strand. All bases were modified using alternating 2′-O-methyl /2′-fluoro modification pattern with additional 14 phosphorothioates incorporated as shown. The 3′ end of the passenger strand was conjugated to a hydrophobic teg-Chol (tetraethylene glycol cholesterol). Combination of described modification enables quick, efficient internalization by primary neurons without requirement for formulation or electroporation.

Standard solid-phase oligonucleotide synthesis.Oligonucleotides were synthesized on an OligoPilot100 Synthesizer following standard protocols. Each synthesis was done at a 50-200-umole scale using Chole-teg or Unylinker terminus (ChemGenes, Wilmington, MA) support. The sequence of the hsiRNA^{HTT} used in this study is shown in the Table S-2 below.

Table S-2

siRNA ID	Accession number	Strand	Sequence
hsiRNA^{NTC}	N/A	Sense	fU#mG#fA.mC.fA.mA.fAm.U.fA.mC.fG.mA.fU#mU#fA-TegChol
		Antisense	PmU#fA#mA.fU.mC.fG.mU.fA.mU.fU.mU.fG.mU#fC#mA#fA#mU#fC#mA#fU
hsiRNA^{HTT}	NM_002111	Sense	fC#mA#fG.mU.fA.mA.fA.mG.fA.mG.fA.mU.fU#mA#fA-TegChol
		Antisense	PmU#fU#mA.fA.mU.fC.mU.fC.mU.fU.mU.fA.mC#fU#mG#fA#mU#fA#mU#fA

Chemical modifications are designated as follows. "." – phosphodiester bond, "#" – phosphorothioate bond, "m" – 2'-OMethyl, "f" – 2'-Fluoro, "P" – 5' Phosphate, "teg-cholesterol" – tetraethylene glycol (teg)-Cholesterol.

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Phosphoramidites were prepared as 0.15 M solutions for 2´-O-methyl (ChemGenes, Wilmington,MA), Cy3 (Gene Pharma, Shanghai, China) and 2´-fluoro (BioAutomation, Irving, Texas) in ACN.5-(Benzylthio)-1H-tetrazole (BTT) 0.25 M in ACN was used as coupling activator. Detritylationswere performed using 3% dichloroacetic acid (DCA) in DCM and capping was done with a 16% N-methylimidazole in THF (CAP A) and THF:acetic anhydride:2,6-lutidine, (80:10:10, v/v/v) (CAP B) for 15 s. Sulfurizations were carried out with 0.1 M solution of DDTT in ACN for 3 minutes. Oxidation was performed using 0.02 M iodine in THF:pyridine:water (70:20:10, v/v/v). Deprotection and purification of oligonucleotides. Both sense and antisense strands were cleaved and deprotected using 40% aq.methylamine at 65 °C for 15 minutes. The oligonucleotide solutions were then cooled in a freezer and dried under vacuum in a Speedvac. The resulting pellets were suspended in water. The final purification of oligonucleotides was performed on an Agilent Prostar System (Agilent, Santa Clara, CA) equipped with a Hamilton HxSil C18 column (150x21.2). The pure oligonucleotides were collected, desalted by size-exclusion chromatography using a Sephadex G25 and lyophilized. LC-MS analysis of oligonucleotides.The identity of oligonucleotides was established by LC-MS analysis on an Agilent 6530 accuratemass Q-TOF LC/MS (Agilent technologies, Santa Clara, CA).

The purified strands were duplexed and duplex formation and purify confirmed by gel.

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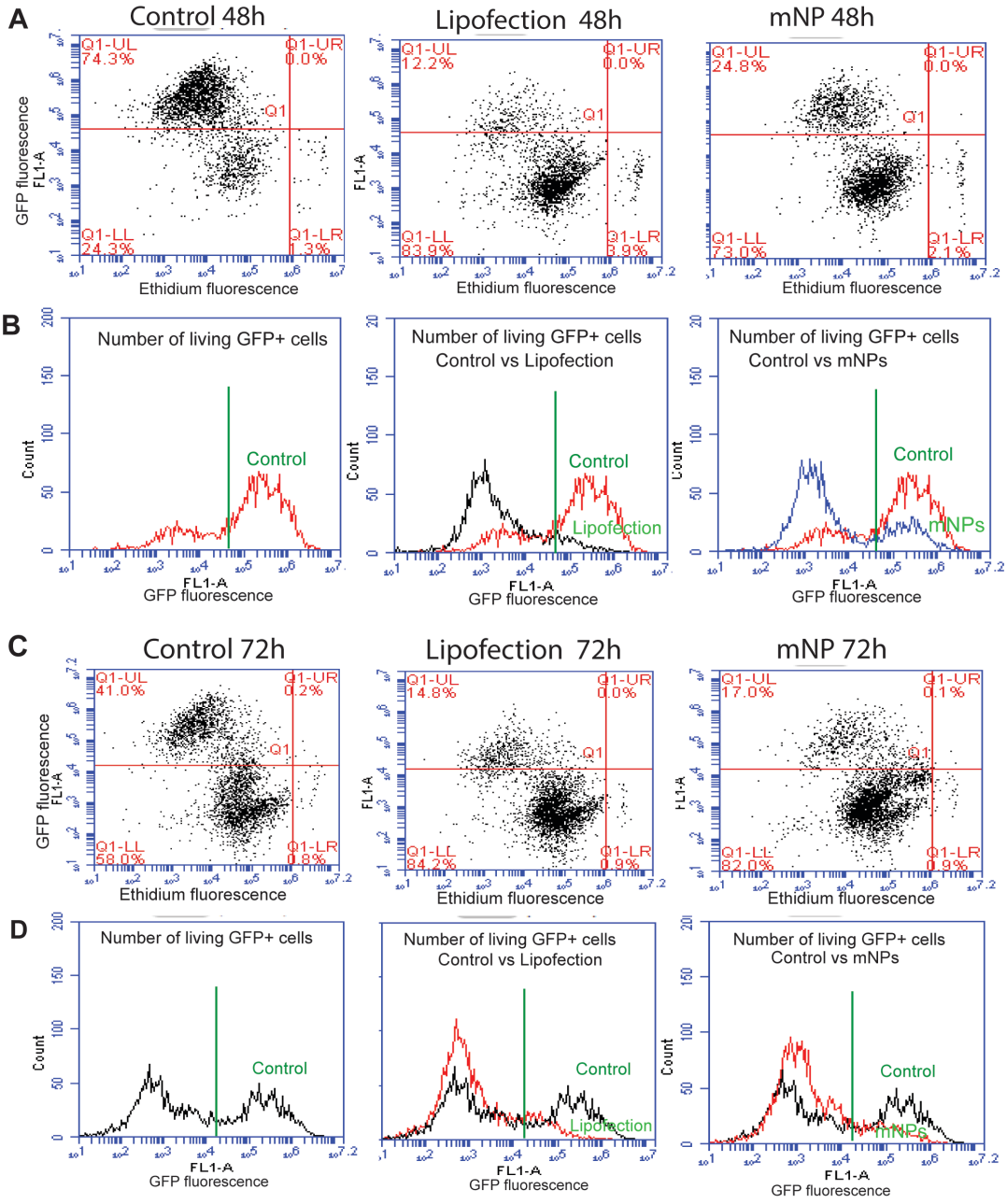

E

	"Living" GFP+ (% of total cells in upper left quadrant)	Dying GFP+ (% of total cells in upper right quadrant)	"Living" GFP- (% of total cells in lower left quadrant)	"dead" cells (% of total cells in lower right quadrant)
Control 48 h	74.3 %	0.04 %	24.3 %	1.28 %
Control 72 h	40.9 %	0.20 %	58.0 %	0.82 %
Lipofection 48 h	12.2 %	0.00 %	83.9 %	3.89 %
Lipofection 72 h	14.8 %	0.00 %	84.2 %	0.95 %
mNP 48 h	24.8 %	0.04 %	72.9 %	2.15 %
mNP 72 h	16.9 %	0.10 %	82,0 %	0.89 %

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Legend for Figure S-**5**

FACS was performed to enumerate GFP+ and ethidium+ cells in eGFP expressing NIHT3 cells. The cell cultures were incubated for 48 or 72 h with either mNPs or lipofectamine bearing anti-GFP siRNA. Ethidium staining was used to identify dying or dead cells. Panels from A and C present FACS data as dot plots showing GFP silencing after 48 and 72 h, respectively. Dot plots indicate the number of cells (expressed as percentage of total cells counted) that are GFP+ and ethidium+ or both. The X-axis indicates ethidium fluorescence intensity and the Y-axis shows GFP fluorescence intensity. In each dot plot panel, cells in the upper left quadrant are GFP+ cells that have no ethidium staining (e.g. living GFP+ cells). The left lower quadrants show living cells that do not express GFP above the threshold. Notice that under control conditions 24% of cells have very low GFP fluorescence at 48 h (Panel A) and 58% at 72 h (Panel C). The right upper quadrants indicate GFP+ cells that also are stained with ethidium (dying GFP+ cells). The right lower quadrant shows ethidum+ cells that do not express GFP (dead cells). Panels B and D show the data as cell counts (Y-axis) plotted against GFP fluorescence intensity. Panel E summarizes the data in numerical form, indicating that mNP cytotoxicity was not significantly different from that elicited by lipofection. Indeed it was slightly less with mNP than with lipofection after 72 h.