Bioconjugate Chem. Supporting Information

CLIP6-PNA-peptide Conjugates: Non-endosomal Delivery of Splice Switching Oligonucleotides (SSO)

Terese Soudah[a], ‡ Maxim Mogilevsky [b], ‡ Rotem Karni [b], Eylon Yavin[a*]

[a]The Institute for Drug Research, The School of Pharmacy, The Faculty of Medicine, The Hebrew University of Jerusalem, Hadassah Ein-Kerem, Jerusalem 91120, Israel.

[b]Department of Biochemistry and Molecular Biology, Institute for Medical Research Israel-Canada, The Faculty of Medicine, The Hebrew University of Jerusalem, Hadassah Ein-Kerem, Jerusalem 91120, Israel.

E-mail: eylony@ekmd.huji.ac.il; Fax:+972-2-6757574; Tel:+972-2-6758692.

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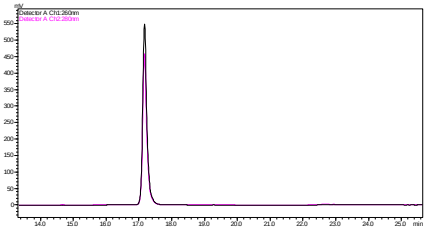
Anti2b-PNA-**NLS**



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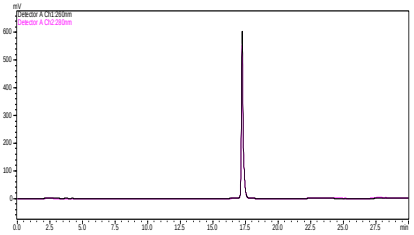

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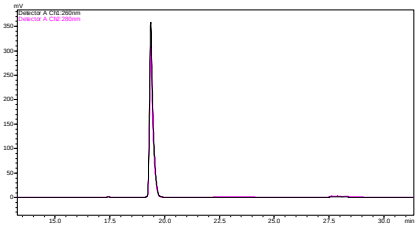

Scr-PNA-**CLIP6**

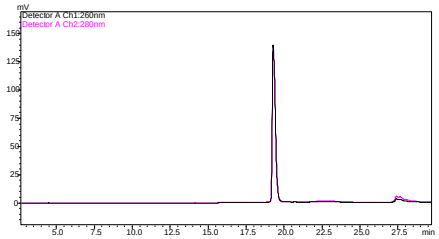

Figure S1. HPLC chromatograms of purified PNA-peptide conjugates. Over 95% purity for all PNA-peptide conjugates.

RP-HPLC (Shimadzu LC2010), semi-preparative C18 reverse-phase column (Phenomenex, Jupiter 300 A) at a flow rate of 4 mL/min. Mobile phase: 0.1% TFA in H2O (A) and acetonitrile (B).
Gradient: Initial −90% A, 10% B. 10 min − 40% A, 60% B. 30 min −10% A, 90% B. 30.01 min −10% A, 90% B. 37 min – 95% A, 5% B. 37.01 min– 95% A, 5% B. 40 min–stop, 44.01min.

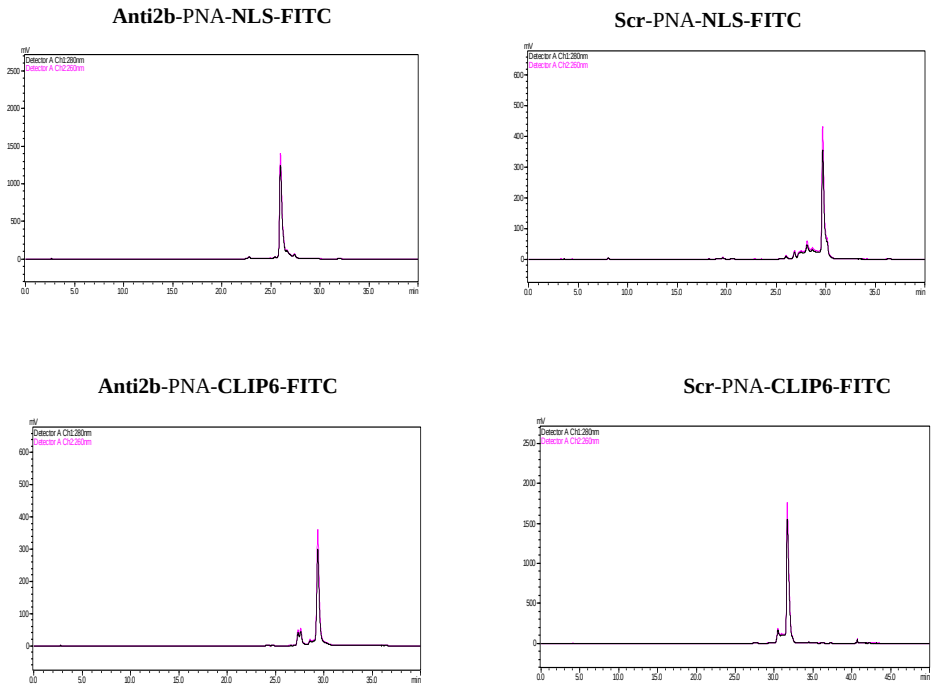

Figure S2. HPLC chromatograms of purified FITC-labeled PNA-peptide conjugates. Purity of FITC-labelled PNA-peptides: 94% for Anti2b-PNA-NLS-FITC; 86% for Scr-PNA-NLS-FITC; 90% for Anti2b-PNA-CLIP6-FITC; 94% for Scr-PNA-CLIP6-FITC.

RP-HPLC (Shimadzu LC2010), semi-preparative C18 reverse-phase column (Phenomenex, Jupiter 300 A) at a flow rate of 4 mL/min. Mobile phase: 0.1% TFA in H2O (A) and acetonitrile (B).
Gradient: Initial −90% A, 10% B. 10 min − 73% A, 27% B. 35 min −10% A, 90% B. 40.01 min −10% A, 90% B. 47 min – 95% A, 5% B. 47.01 min– 95% A, 5% B. 50 min–stop, 54.01 min.

Primer sequences

Gene name	**Exon forward** **Exon reverse**	**Sequence (5'–3')**
MKNK2	e12 For	GCTGCGACCTGTGGAGCCTGGG
	e14a Rev	GATGGGAGGGTCAGGCGTGGTC
	e14b Rev	GAGGAGGAAGTGACTGTCCCAC
GAPDH	For	ATCAAGAAGGTGGTGAAGCAG
	Rev	CTTACTCCTTGGAGGCCATGT

Table S1: primers used for RT-PCR presented in Figure 1 and Figure S4.

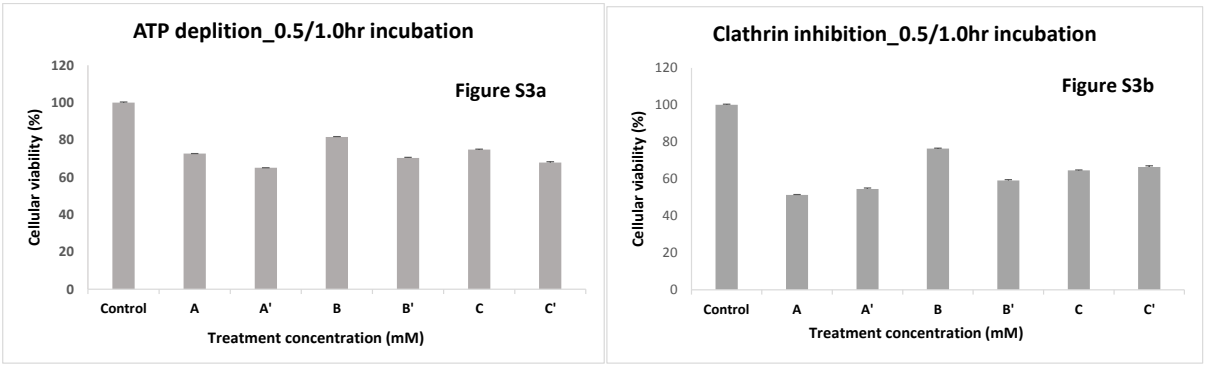

Figure S3: MTT Assay-cells treated under ATP depletion and hyperosmolar sucrose conditions

Figure S3a
Control U87-MG glioblastoma cells: no treatment
A/A'-cells: treated with endocytosis inhibition reagents (10mM sodium azide + 50mM 2-deoxy-D-glucose) for 0.5hr/1.0hr
B/B'- cells: treated with endocytosis inhibition reagents (5mM sodium azide + 25mM 2-deoxy-D-glucose) for 0.5hr/1.0hr
C/C'- cells: treated with endocytosis inhibition reagents (2.5mM sodium azide + 12.5mM 2-deoxy-D-glucose) for 0.5hr/1.0hr

Figure S3b.
Control U87-MG glioblastoma cells: no treatment
A/A'-cells: treated with endocytosis inhibition reagents (0.45M Sucrose) for 0.5hr/1.0hr
B/B'- cells: treated with endocytosis inhibition reagents (0.22M Sucrose) for 0.5hr/1.0hr
C/C'- cells: treated with endocytosis inhibition reagents (0.11M Sucrose) for 0.5hr/1.0hr

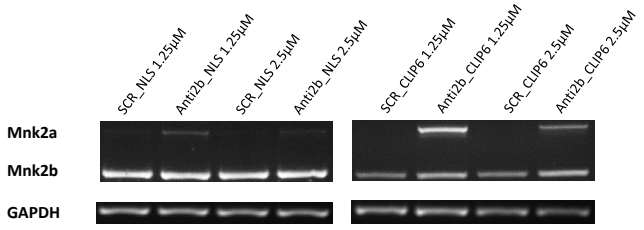

Figure S4. Repeated gel - Mnk2a isoform formation by Anti2b-PNA-NLS and Anti2b-PNA-CLIP6 SSOs. U87MG cells were incubated with 1.25 and 2.5μM Anti2b-CLIP6-PNA, Anti2b-NLS-PNA or scrambled control PNAs. RT-PCR was determined after 72 hours.