

**An anti-miR-155 cyclic peptide-PNA conjugate: synthesis,  
cellular uptake, and biological activity**

Terese Soudah<sup>a</sup>, Saleh Khawaled<sup>b</sup>, Rami I. Aqeilan<sup>b</sup>, Eylon Yavin<sup>\*a</sup>

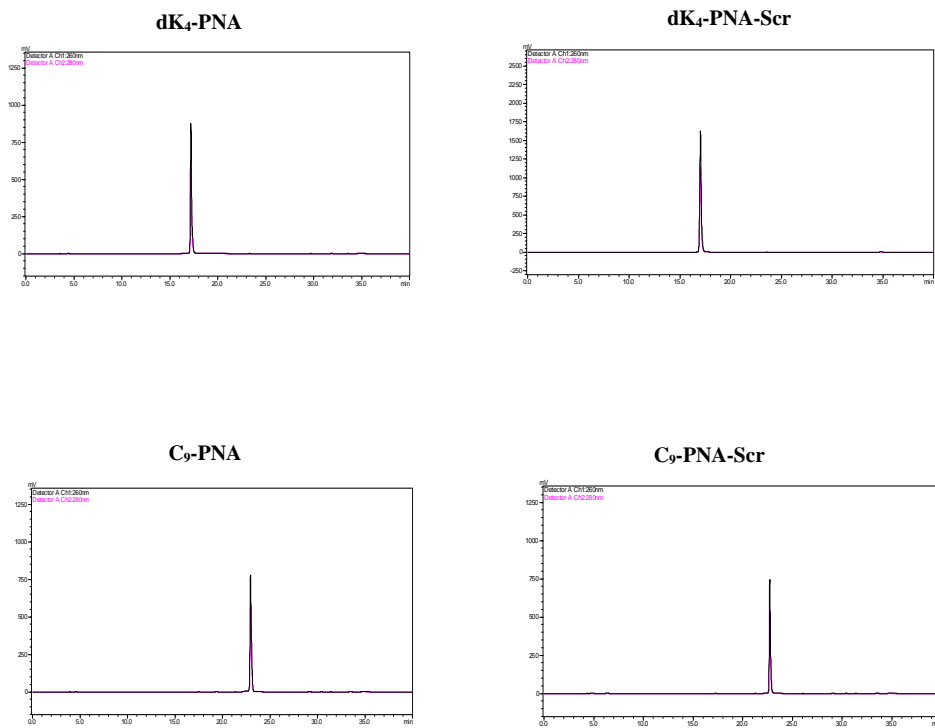
<sup>a</sup>The Institute for Drug Research, The School of Pharmacy, The Hebrew University of Jerusalem, Hadassah Ein-Kerem, Jerusalem 9112102, Israel.

<sup>b</sup>Lautenberg Center for Immunology and Cancer Research, Institute for Medical Research Israel-Canada, The Hebrew University of Jerusalem, Hadassah Ein-Kerem, Jerusalem 9112102, Israel.

E-mail: [eylony@ekmd.huji.ac.il](mailto:eylony@ekmd.huji.ac.il); Fax: +972-2-6757574; [Tel: +972-2-6758692](tel:+972-2-6758692).

**Tables of contents:**

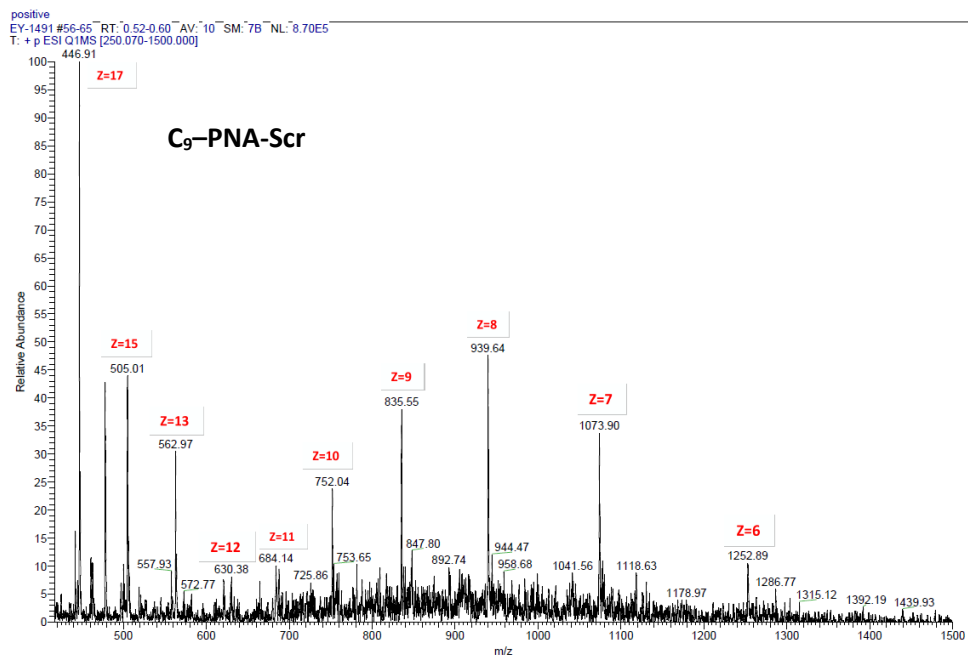
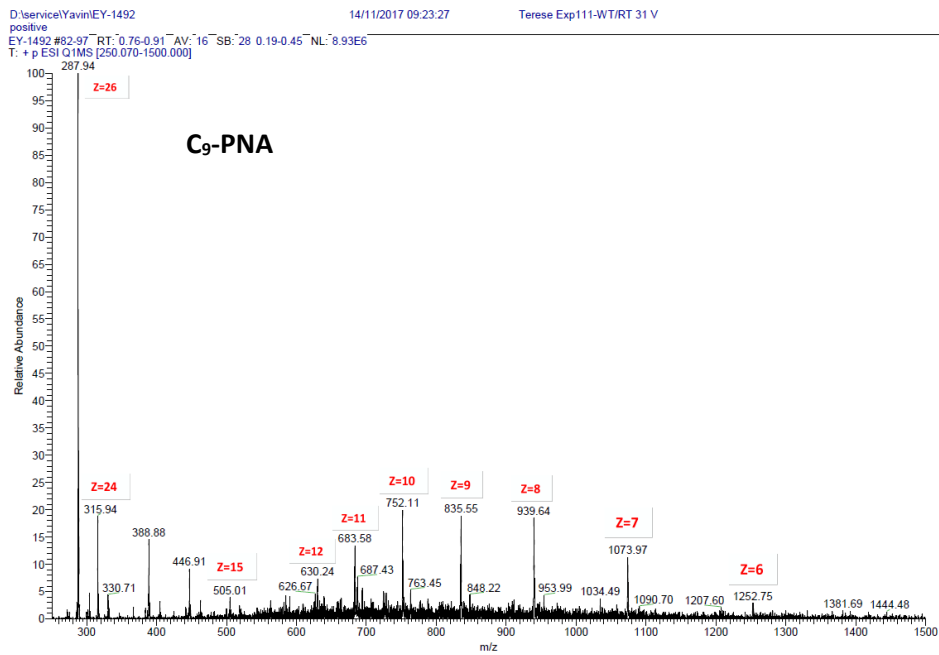
|  |           |     |
|--|-----------|-----|
| HPLC chromatograms of purified PNA-peptide conjugates                      | Figure S1 | S3  |
| Mass Spectra of purified C <sub>9</sub> -PNA and C <sub>9</sub> -PNA-Scr   | Figure S2 | S4  |
| Mass Spectra of purified dK <sub>4</sub> -PNA and dK <sub>4</sub> -PNA-Scr | Figure S3 | S5  |
| HPLC chromatograms of crude FITC-labelled PNA-peptide conjugates           | Figure S4 | S6  |
| HPLC chromatograms of purified FITC-labelled PNA-peptide conjugates        | Figure S5 | S7  |
| Mass Spectra of purified FITC-labelled PNA-peptide conjugates              | Figure S6 | S8  |
| Primer sequences   | Table S1  | S9  |
| Cross Section Confocal Images - C <sub>9</sub> -PNA-FITC                   | Figure S7 | S10 |
| Cross Section Confocal Images - C <sub>9</sub> -PNA-FITC co-stained        | Figure S8 | S10 |
| XTT of Nf08-uterus normal cells  | Figure S9 | S11 |



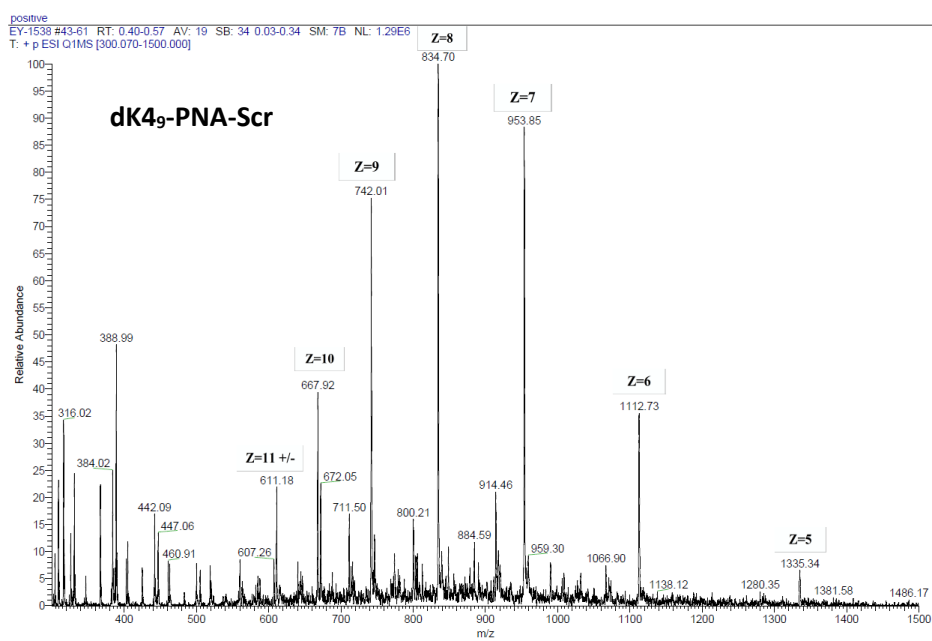
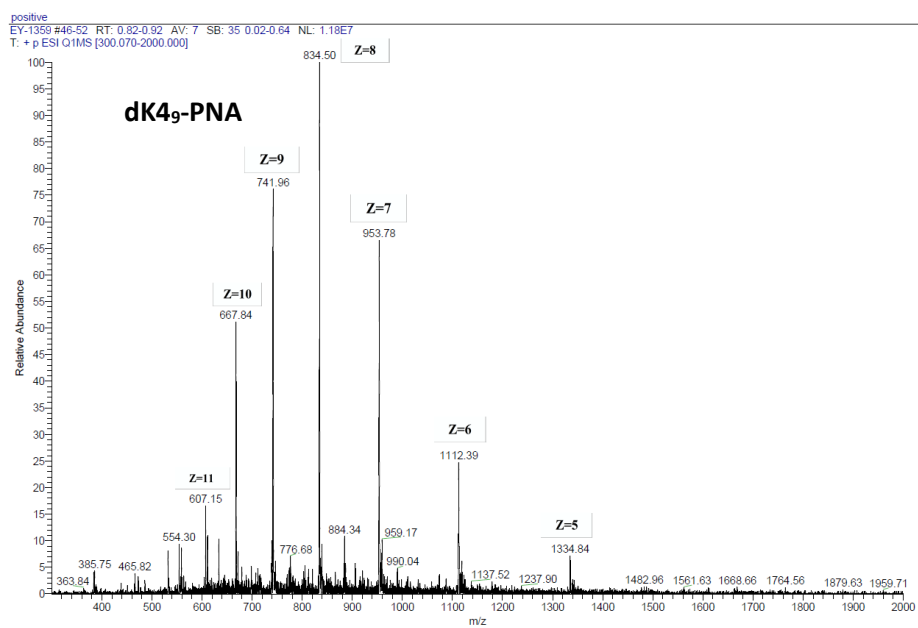
**Figure S1.** HPLC chromatograms of purified PNA-peptide conjugates. Over 95% purity for all PNA-peptide conjugates.

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 H<sub>2</sub>O (A) and acetonitrile (B).

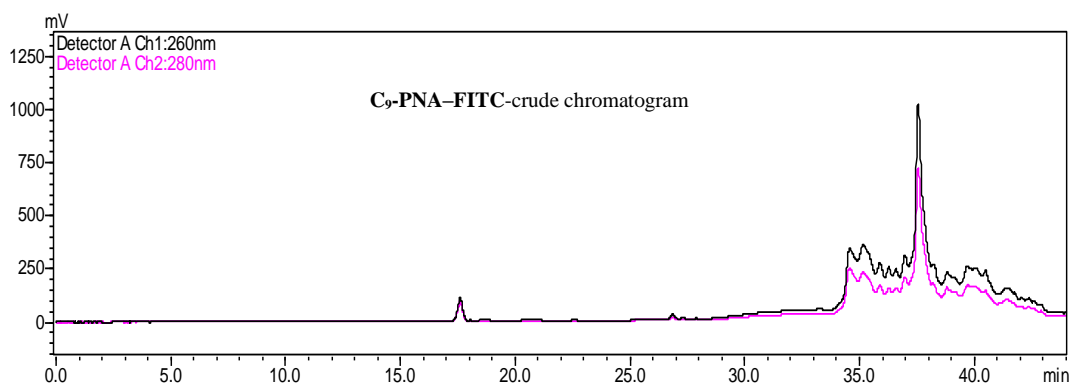
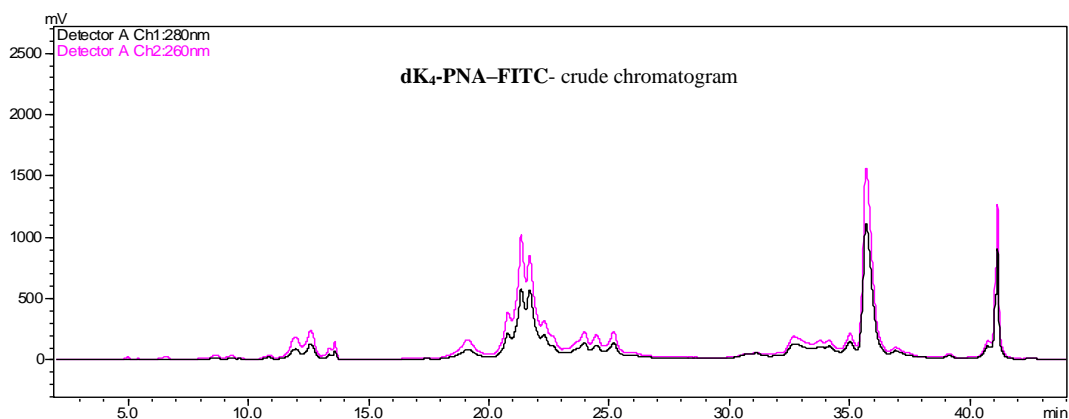
Gradient: Initial –90% A, 10% B. 10 min – 40% A, 60% B. 30 min –10% A, 90% B. 30.01 min –10% A, 90% B. 37 min – 95% A, 5% B. 37.01 min– 95% A, 5% B. 40 min–stop, 44.01 min.



**Figure S2.** Mass spectra (ESI) of C<sub>9</sub>-PNA and C<sub>9</sub>-PNA-Scr.



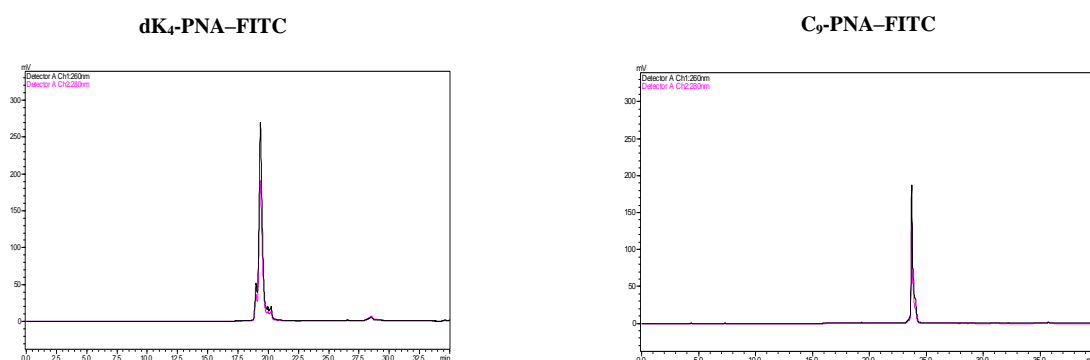
**Figure S3.** Mass spectra (ESI) of dK<sub>4</sub>-PNA and dK<sub>4</sub>-PNA-Scr.



**Figure S4.** HPLC chromatograms of crude FITC-labeled PNA-peptide conjugates. Purity of FITC-labelled PNA-peptides were 22% for dK<sub>4</sub>-PNA-FITC ( $R_t = 35.9'$ ) and 36% for C<sub>9</sub>-PNA-FITC ( $R_t = 36.8'$ ) based on the ratio between the area under curve (AUC) of product peak divided to total AUC (at 260 nm).

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 H<sub>2</sub>O (A) and acetonitrile (B).

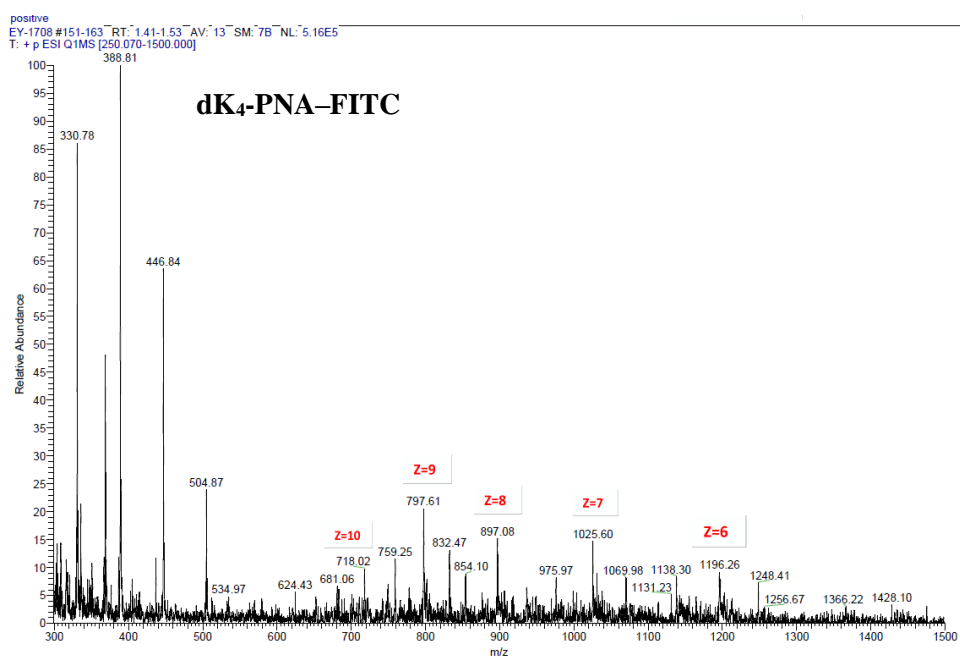
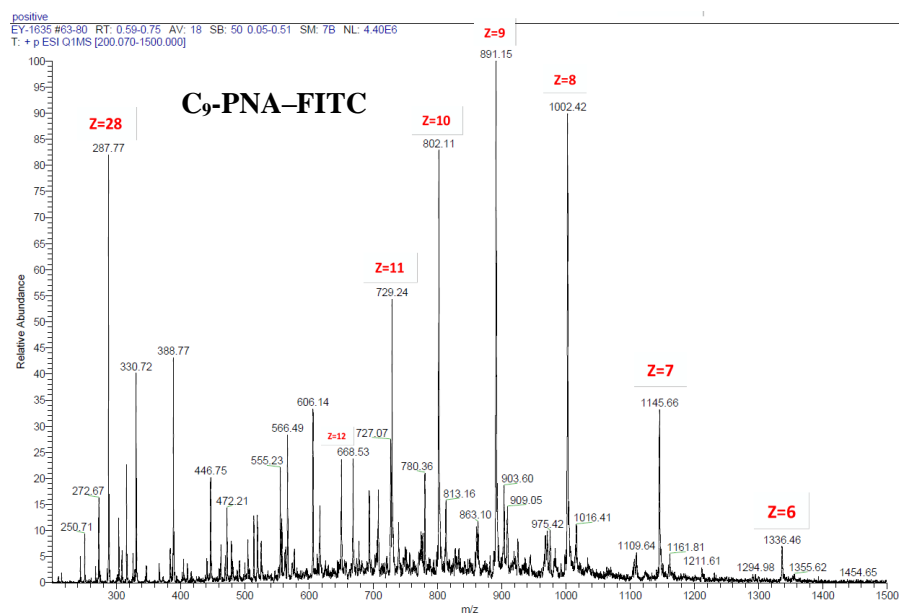
Gradient: Initial –90% A, 10% B. 10 min – 80% A, 20% B. 30 min –10% A, 90% B. 30.01 min –10% A, 90% B. 37 min – 95% A, 5% B. 37.01 min– 95% A, 5% B. 40 min–stop, 44.01min.



**Figure S5.** HPLC chromatograms of purified FITC-labeled PNA-peptide conjugates. Purity of FITC-labelled PNA-peptides: 91% for dK<sub>4</sub>-PNA-FITC and 93% for C<sub>9</sub>-PNA-FITC, respectively.

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 H<sub>2</sub>O (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.



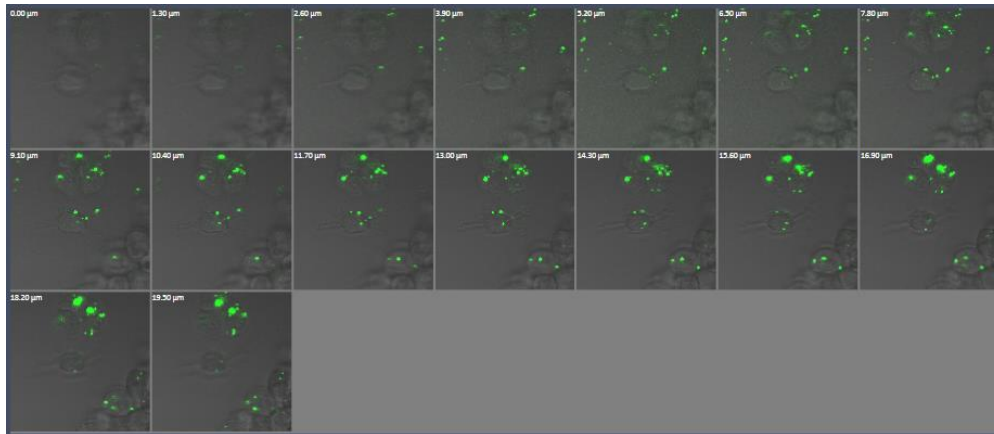
**Figure S6.** Mass spectra (ESI) of C<sub>9</sub>-PNA-FITC and dK<sub>4</sub>-PNA-FITC.



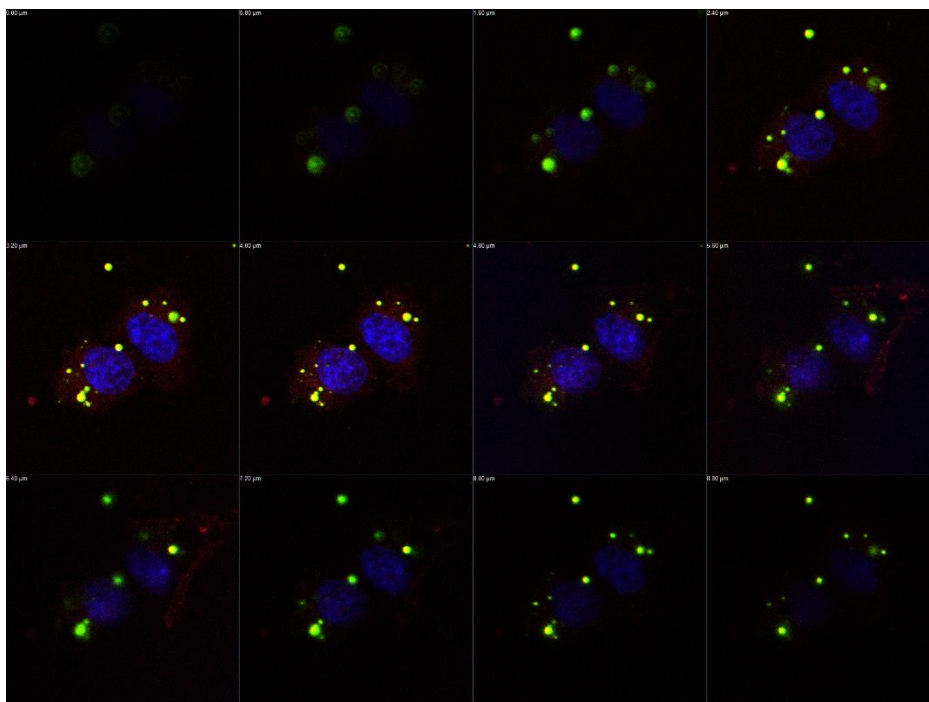
### Primer sequences

| Gene name    | Forward<br>reverse      | Sequence (5'-3')      |
|--------------|-------------------------|-----------------------|
| <i>h UBC</i> | For                     | ATTGGGTCGCGGTTCTTG    |
|              | Rev                     | TGCCTTGACATTCTCGATGGT |
| miR-155-5p   | TTAATGCTAATCGTGATAGGGGT |                       |

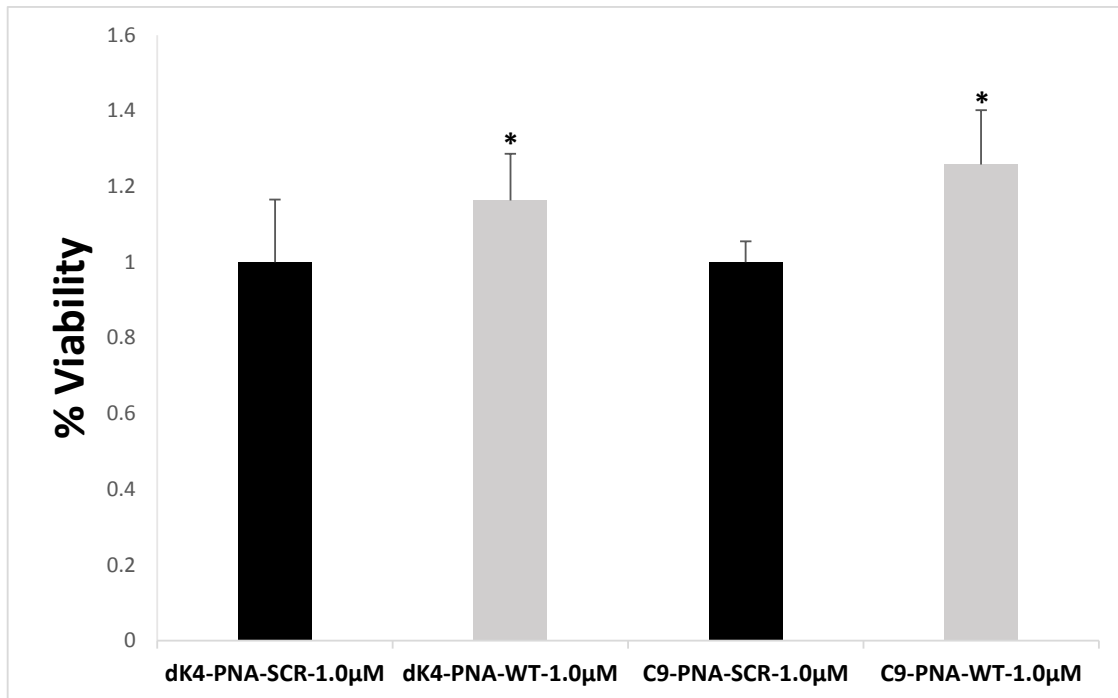
**Table S1:** primers used for RT-PCR presented in Figure 1.



**Figure S7.** Cross section of a confocal image taken for C<sub>9</sub>-PNA-FITC added to U87 cells (0.5 µM, 3 h). Sections are shown with increments of 1.3 microns ranging from 0 to 19.5 microns.



**Figure S8.** Cross section of a co-stained confocal image taken for C<sub>9</sub>-PNA-FITC added to U87MG cells (0.5 µM, 3 h). Sections are shown with increments of 0.8 micron ranging from 0 to 8.8 microns.



**Figure S9.** Cell viability for Nf08-uterus cells as determined by the XTT assay. Nf08-uterus cells were treated with 1  $\mu$ M PNA conjugates for 72 hours at 37  $^{\circ}$ C (in triplicates in 96-well plates). Viability is shown in comparison to scrambled PNA controls. \* *P*-value < 0.05.