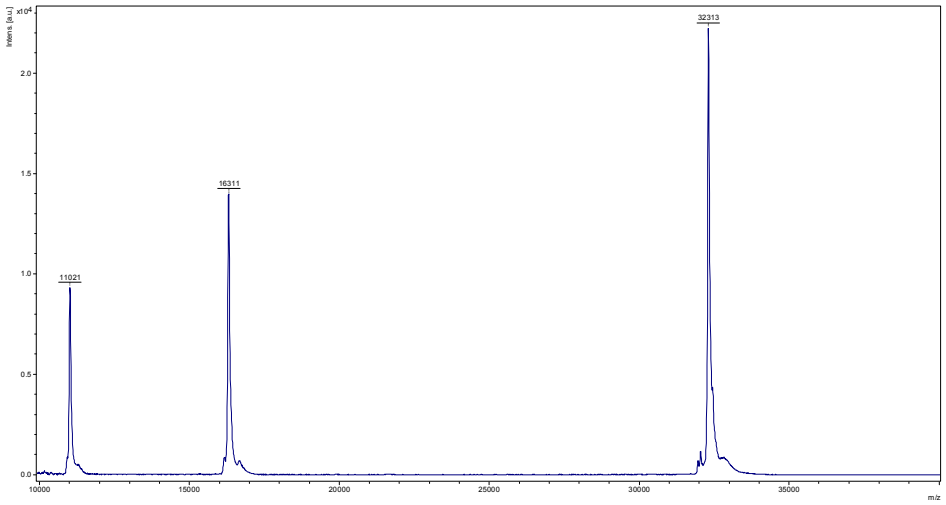
Supporting Information Available

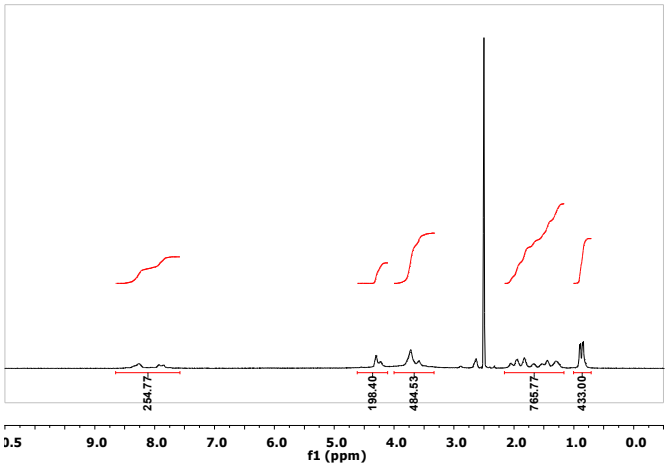
This Supporting Information includes characterization of the ELR by MALDI-TOF, NMR, SDS-PAGE and amino-acid analysis. It is supplemented with the assays performed to determine the influence of pDNA, temperature and pH on the ELR-pDNA polyplexes in terms of condensation ability, transition temperature (Tt), particle size and DNA protection. Additionally two transfection assays and a scheme for z-series and z-stack figure are also incorporated.

ELR POLYMER CHARACTERIZATION

A



B



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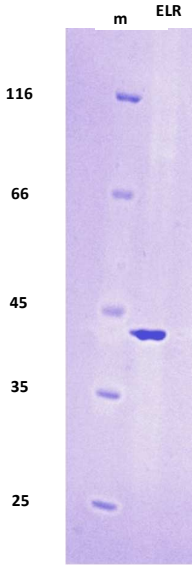


D

Amino acid	Predicted	Experimental
Glu	1	0.82

Ser	1	0.73
Gly	144	148.64
Val	73	74.68
Met	1	0.52
Leu	2	1.83
Lys	72	75.65
Pro	73	74.09

Figure S1. Characterization of the ELR. (A) MALDI-TOF spectra. The two peaks indicated correspond to the singly (32,313 Da) and doubly charged ions (16,311 Da), whereas the expected molecular weight is 32,368 Da. (B) Nuclear magnetic resonance spectra: ^{1}H-RMN (400 MHz, $(CD_3)_2SO$): δ 8.44-8.23 and 8.18-7.88 (288H, br, -*NH*), 4.45-4.24 and 3.67-3.50 (792H, br, *α-CH- Val* + *α-CH- Pro* + *α-CH- Lys* + *-CH_2-Gly* + δ*CH_2-Pro* and *ε–CH_2-Lys*), 2.14-1.64 and 1.60-1.35 (792H, m, *$CH(CH_3)_2$-Val*, *-β* and *γ–CH_2-Pro*, and *β, χ.* and *δ –CH_2-Lys*), 0.97-0.75 (432H, d, *$CH(CH_3)_2$-Val*). (C) SDS-PAGE. The apparent MW corresponds to 42,500 Da. The difference in migration pattern in comparison with the MALDI-TOF result is attributed to the abundance of positively charged amino acids, which cause the polymer to migrate more slowly due to a reduced charge-to mass ratio, thus resulting in a higher apparent MW. This retard effect on electrophoretic mobility for ELRs when compared to a commercial MW marker in SDS-PAGE is well known and was previously described[1-3]. (D) Amino acid

composition analysis. The amino-acid analysis showed an appropriate composition and the differences were attributed to the experimental error of the technique.

BEHAVIOUR OF ELR IN THE PRESENCE OF pDNA

CONDENSATION ABILITY AND STABILITY ASSAY

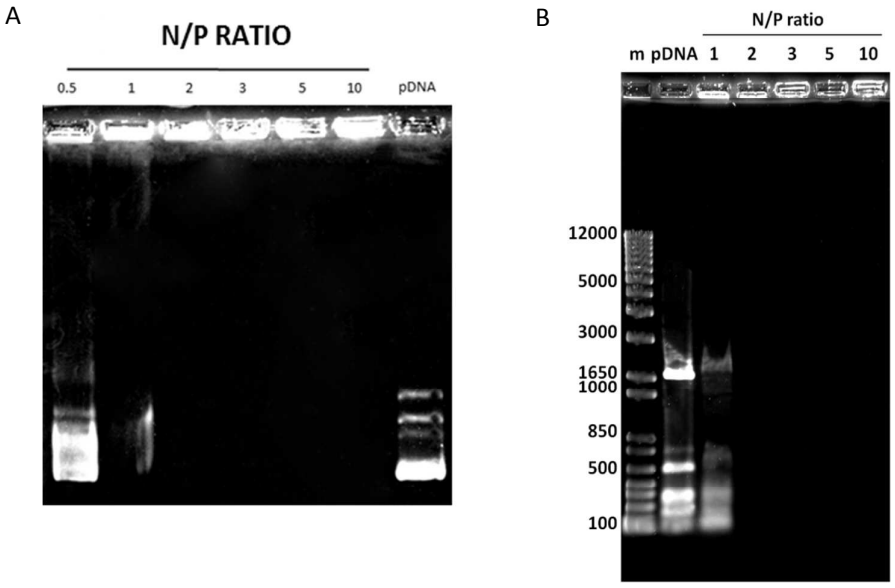


Figure S2. Condensation ability of ELR and stability of polyplexes. Polyplexes were formed at 0.5/1, 1/1, 2/1, 3/1, 5/1, 10/1 N/P ratios and loaded on an agarose gel for electrophoresis. Polymer free pDNA was used as control. The ELR was able to retain the pDNA in the lane from N/P ratio of 2 in comparison with the previous 0.5 and 1 N/P ratio and pDNA alone (A). Polyplexes were formed at 1/1, 2/1, 3/1, 5/1, 10/1 N/P ratios. pDNA and polyplexes were treated with *Dpn* I for 90 minutes and then loaded on an agarose gel for electrophoresis in TAE buffer (B). Polymer-free pDNA was used as control (pDNA) and 1 μg of 1Kb Plus DNA leader as marker (m).

TRANSITION TEMPERATURE

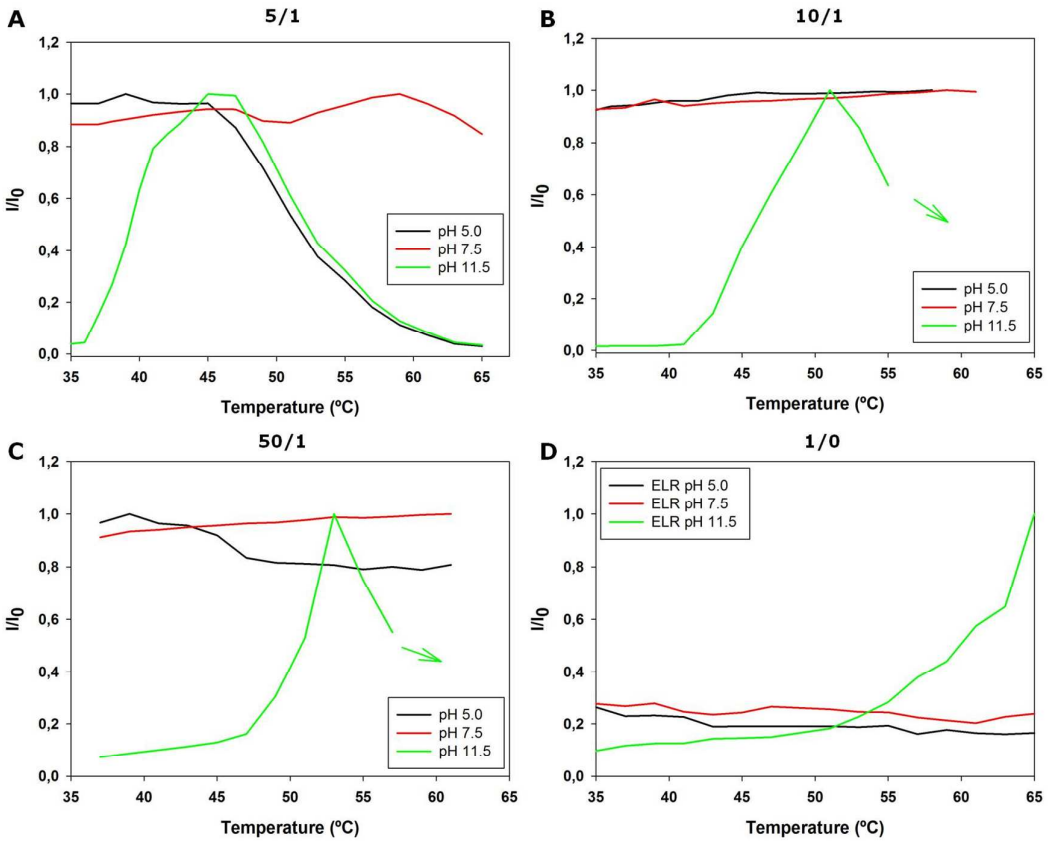
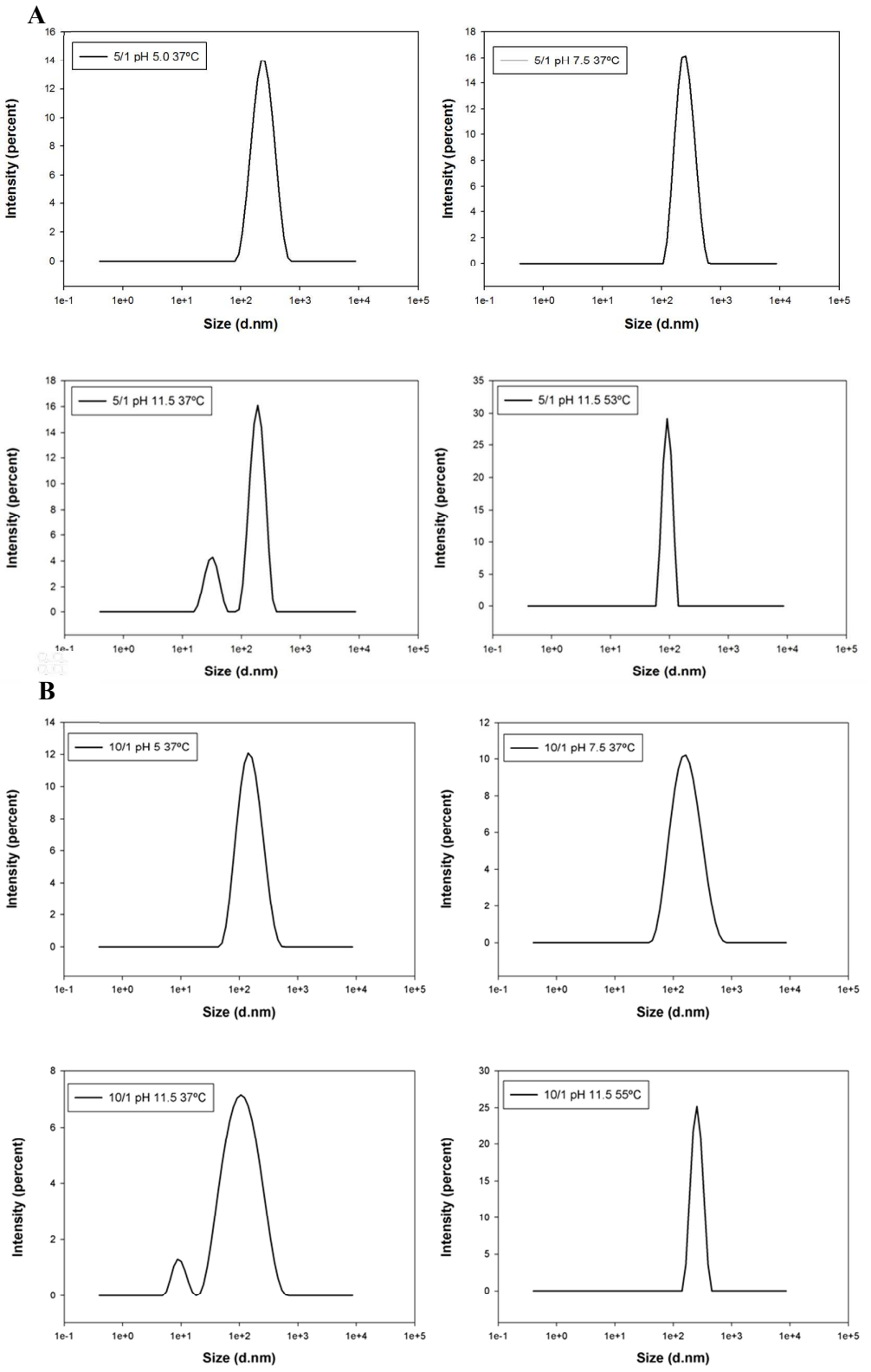


Figure S3. Normalized intensity of scattered light as a function of temperature for polyplexes formed by 1.05 µM, 2.10 µM and 10.5 µM of ELR at different N/P ratios with a constant pDNA concentration of 1.4 nM and 10.5 µM of ELR for 1/0 ratio at different pH. Measurements were performed at pH 5.0, 7.5 and 11.5. Arrows at 10/1 and 50/1 N/P ratios indicate the decreasing tendency in scattered light as a result of the presence of micro-aggregates.

EFFECT OF TEMPERATURE AND pH ON POLYPLEX SIZE



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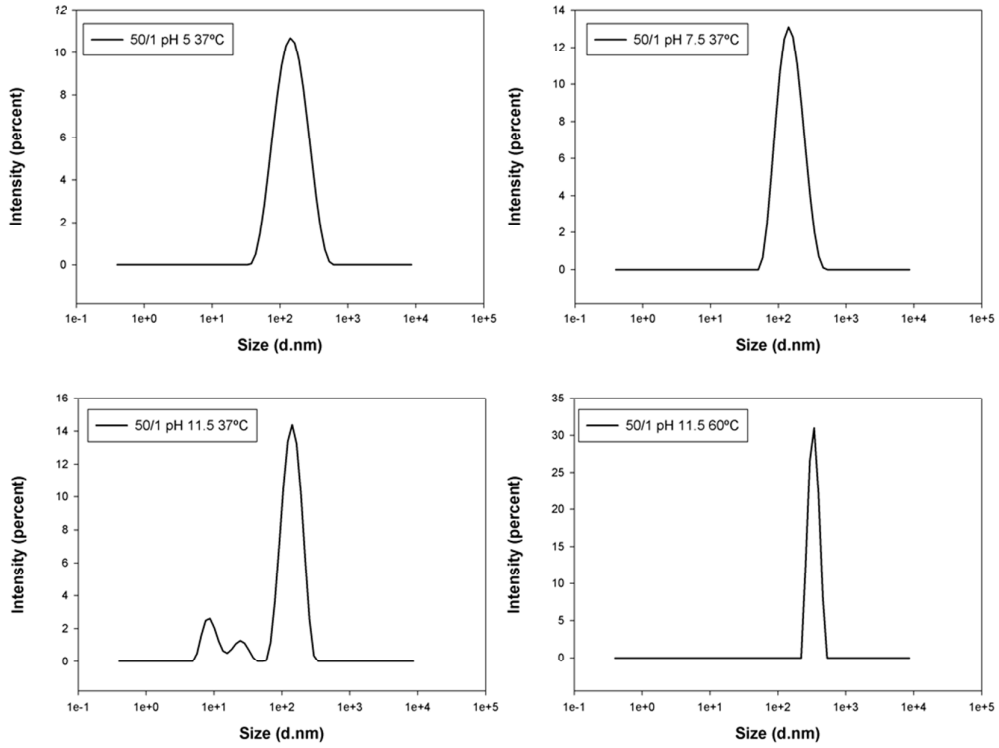

Figure S4. Intensity of scattered light (percent) as a function of temperature at different pH for 1.05 μM, 2.10 μM and 10.5 μM ELR in ELR-pDNA polyplexes at different N/P ratios with a constant pDNA concentration of 1.4 nM. Measurements were performed at pH 5.0, 7.5 and 11.5. (A) N/P ratio of 5/1, (B) N/P ratio of 10/1 and (C) N/P ratio of 50/1.

TRANSFECTION ASSAYS

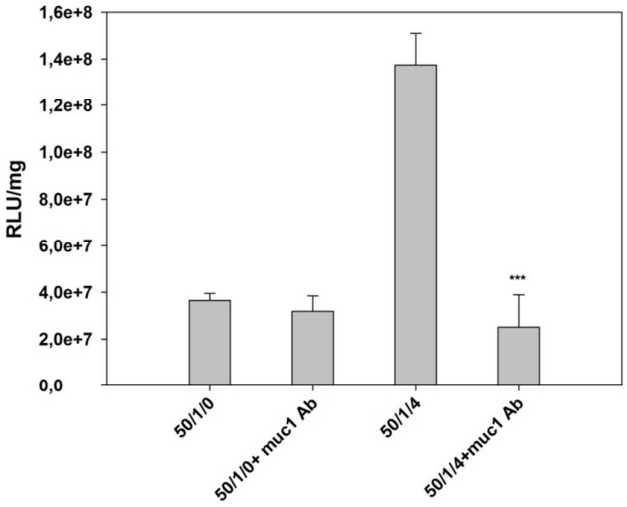

Figure S5. Luciferase expression by pCMV-Gaussia luciferase contained in ELR polyplexes in presence of anti-MUC1 antibody. Polyplexes with 50/1/4 and 50/1/0 without 5TR1 aptamer were incubated in MCF-7 cells pre-treated with anti-MUC1 (muc1 Ab) at 50:1 dilution. Luciferase activity is expressed in RLU/mg protein lysate. The results are expressed as mean±standard error of three independent experiments: $p<0.001$***.

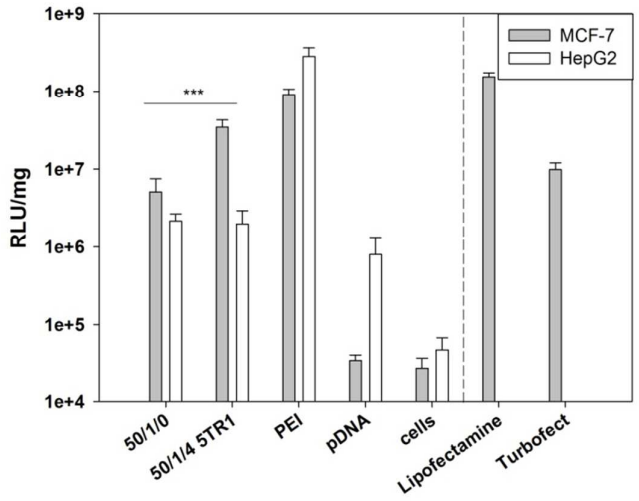

Figure S6. Luciferase expression by pCMV-Gaussia luciferase contained in ELR polyplexes compared with commercial transfection systems. Polyplexes with (50/1/4) and without (50/1/0) 5TR1 aptamer and PEI were incubated in MCF-7 and HepG2 cells. Additionally Lipofectamine LTX and Turbofect were incubated with MCF-7 cells. Luciferase activity is expressed in RLU/mg protein lysate. The results are expressed in logarithmic scale as mean±standard error of three independent experiments. ***: $p<0.001$.

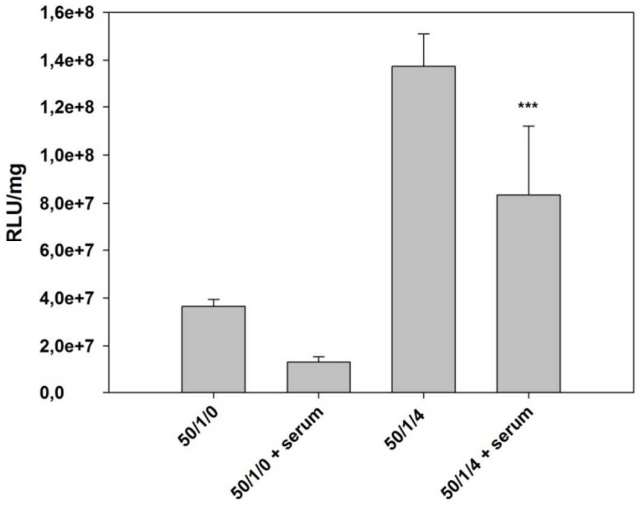

Figure S7. Luciferase expression by pCMV-Gaussia luciferase contained in ELR polyplexes in presence of 10% of serum. Polyplexes with (50/1/4) and without (50/1/0) 5TR1 aptamer were incubated in presence or absence of serum in MCF-7 cells. Polyplex without serum was used as control. Luciferase activity is expressed in RLU/mg protein lysate. The results are expressed as mean±standard error of three independent experiments. ***: $p<0.001$.

Z-SERIES SUCCESSION SCHEME

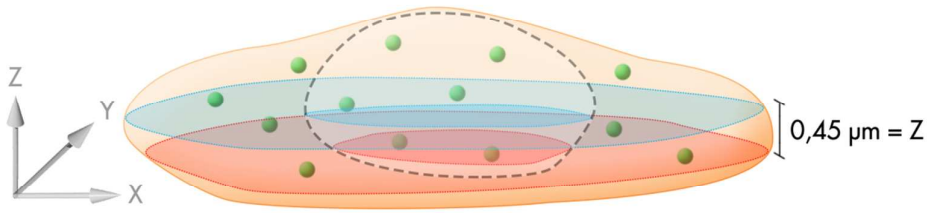

Figure S8. Z-series succession scheme showing the different focal planes showed in Figure 6C and D accomplished by fluorescence microscopy.

Z-STACK

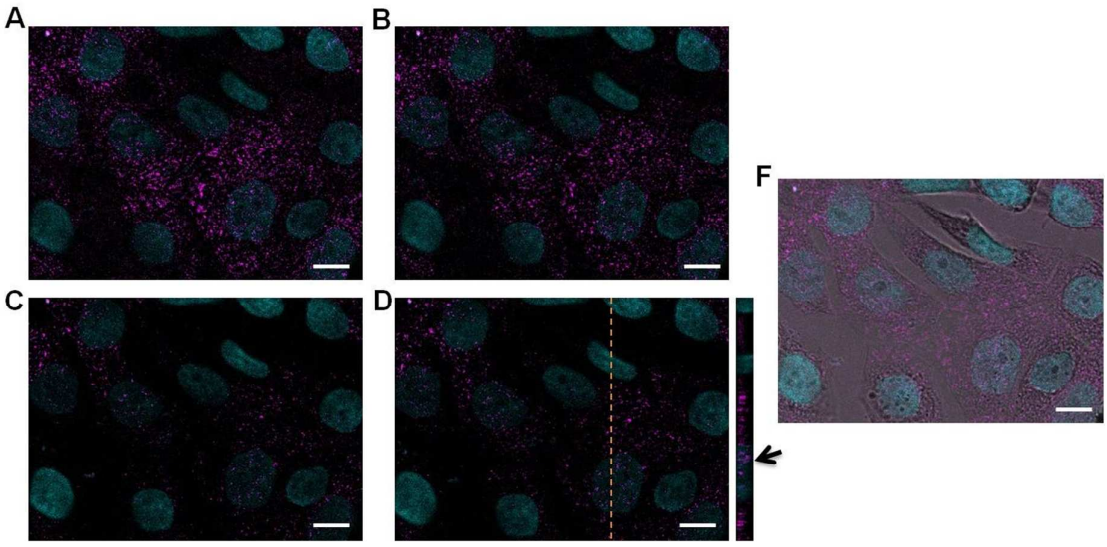

Figure S9. Z-stack (A-D) and orthogonal projection of z-stacks in the z-y planes (D) for MCF-7 cells incubated with ELR-pDNA-5TR1 polyplexes for 3 hours. The z-focal difference between images from A to D is 0.2 µm. Nucleus that appears in light blue has been stained with DAPI and pDNA from polyplexes that is shown in pink has been stained with fluorescein. Light blue and pink colors have been chosen in order to

facilitate its visualization. Merged of DAPI, Fluorescein and phase contrast in B position was performed in order to visualize the healthy state of cells (F). pDNA could be observed in cytoplasm but also co-localized in nucleus (black arrow in D). Scale bar corresponds with 10μm.

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1. Meyer, D. E.; Chilkoti, A. Genetically encoded synthesis of protein-based polymers with precisely specified molecular weight and sequence by recursive directional ligation: Examples from the elastin-like polypeptide system. *Biomacromolecules* **2002,** *3*, (2), 357-367.
2. McPherson, D. T.; Morrow, C.; Minehan, D. S.; Wu, J.; Hunter, E.; Urry, D. W. Production and purification of a recombinant elastomeric polypeptide, G-(VPGVG)19-VPGV, from Escherichia coli. *Biotechnol Prog* **1992,** *8*, (4), 347-52.
3. Trabbic-Carlson, K.; Setton, L. A.; Chilkoti, A. Swelling and mechanical behaviors of chemically cross-linked hydrogels of elastin-like polypeptides. *Biomacromolecules* **2003,** *4*, (3), 572-580.