Supplemental Material for

Polyplex-Induced Cytosolic Nuclease Activation leads to Differential Transgene Expression

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Figure S1. HeLa Cell Viability after 3-hour transfection incubation. Polyplex preparation: 40,000 cells/ well were plated and incubated overnight in 96-wells plate. Wells were rinsed with PBS containing Ca^{2+} and Mg^{2+}. 10 µL Polyplexes at N:P ratio 5:1, 10:1 and 20:1 containing 0.08 µg of Molecular Beacon were then added to 90 µL SFM. 3 hours post-transfection media was removed and added to a replicate 96 wells plate. The media was then used for the LDH assay and the cells were used for the XTT assay. XTT assay: Cells were rinsed with PBS containing Ca^{2+} and Mg^{2+}. 50 µL of PBS containing divalent ions was then added to cells and followed by 50 µL XTT working solution. The plate was then incubated at 37 °C for 4 hours before it was measured. LDH assay: 50 µL of supernatant was incubated with 50 µL of the LDH working solution. The plate was then incubated at 37 °C for 20 minutes and then measured.

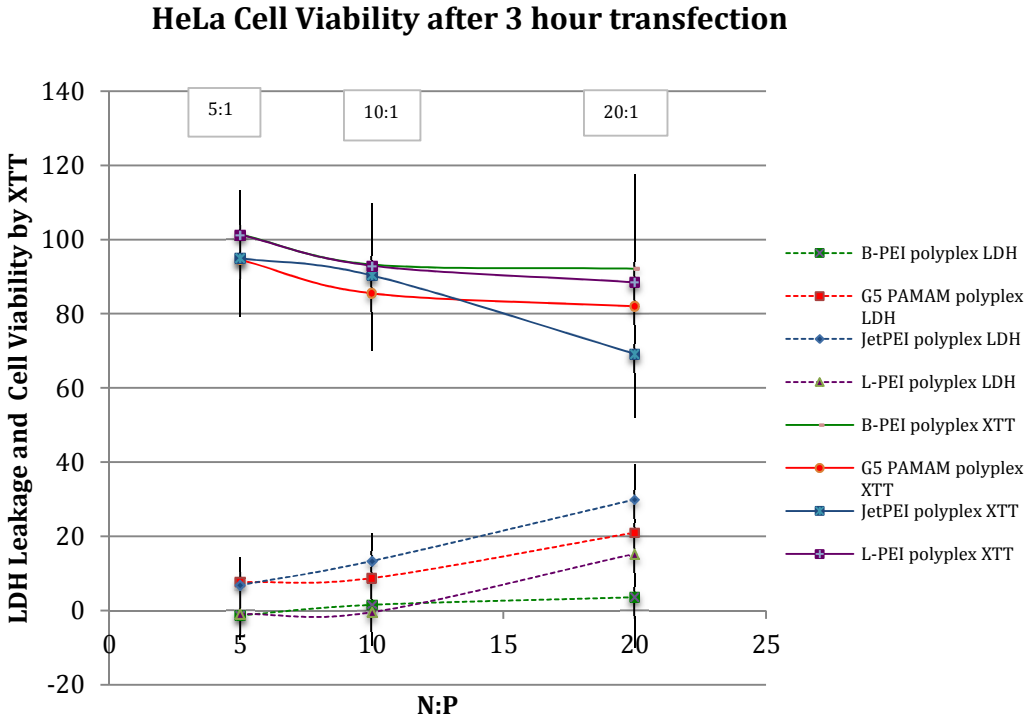


Figure S2. Molecular Beacon stability analysis. The experiment was carried out in a 96-well plate. Each well contained 0.08 µg of molecular beacon diluted in10 µL water solution. Molecular beacon solution was then added to wells containing 90 µL of water or PBS at pH 2, 5 and 7.4 without S1 nuclease or in S1 nuclease buffer with S1 nuclease. The plate was read at excitation of 485/20 nm and emission of 528/20 nm. Based on this study it can be concluded that molecular beacon is stable at acidic and neutral pH.

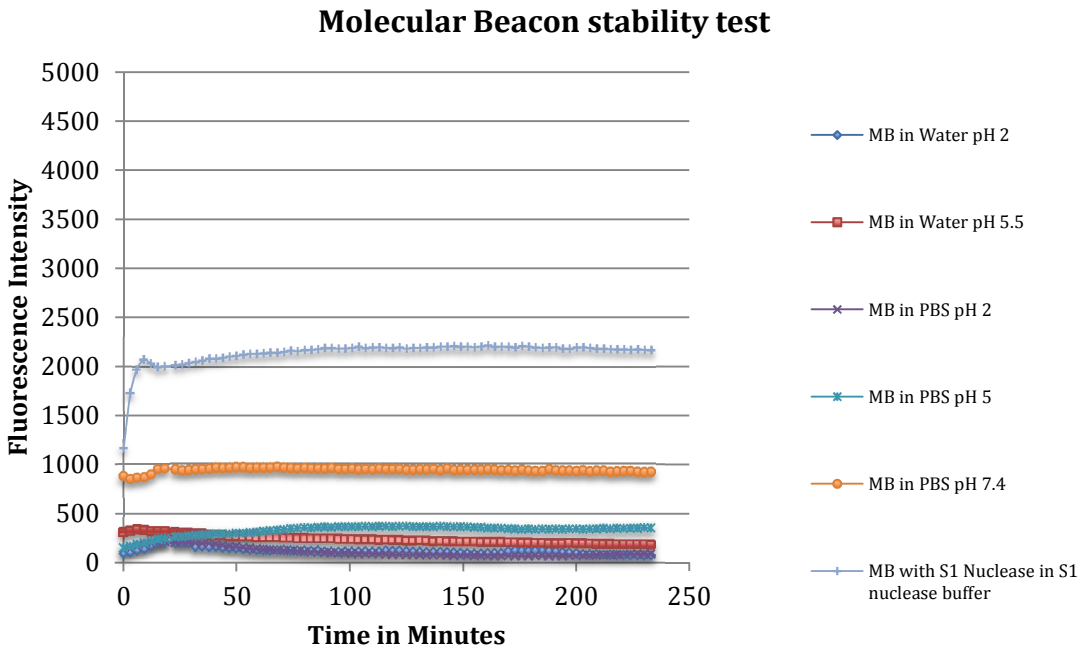
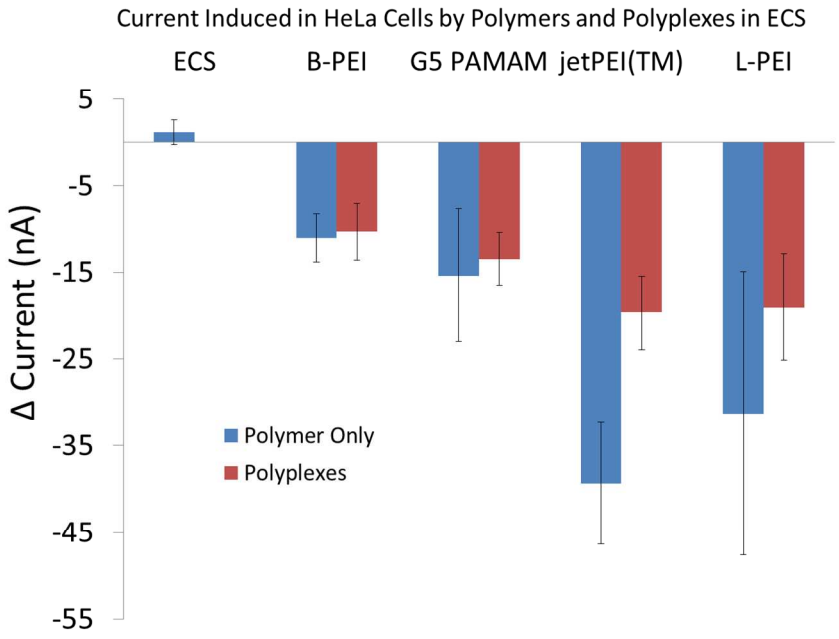


Figure S3. Cell Membrane Permeability Induced by Polyplex Exposure in ECS.

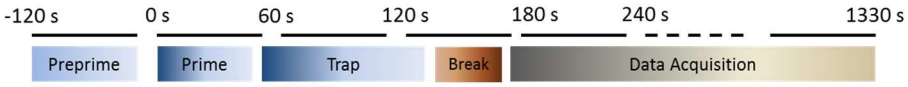


Operation of IonFlux 16TM for whole-cell patch clamp analysis.

The IonFlux 16™ consists of a 96 well microwell plate etched with microfluidic channels at the plate bottom as previously described by Ionescu-Zanetti et al.[1] Each plate is divided into 8 patterns containing two zones that trap 20 cells each. The current trace obtained by each electrode is the combined current from 20 cells. Each pattern has eight wells for compound addition that are independently controlled pneumatically. Each pattern has an IN well which is loaded with cells suspended in ECS. Before the actual experiment, the plate is "preprimed" for two minutes. During the preprime step, the IN well is filled with ECS. The trap wells are filled with ICS and the compound wells have compound solutions. After the preprime step, the IN well is filled with cells suspended in ECS. The experiment consists of four phases: Prime, Trap, Break and Data Acquisition. The timeline and pressure settings for the four phases are provided in supplementary figure S4. In the trap phase, the fluid in the main channel flows in pulses (no flow for 4.2, followed by fluid flowing for 0.8 s), which allows cells to be trapped. The trapped cells are perforated using a rectangular pressure pulse of amplitude 4 psi for 10 s during the break phase. During the data acquisition phase, the main channel and the trap pressures are constant at 0.16 psi and 6 mm Hg respectively. The voltage for data acquisition was set at -70 mV and the sampling rate was 500 Hz. The data was collected in frames that lasted 30 s followed by a 0.3 s period when no data was collected. Once the cells were trapped, they were exposed to ECS for 65 s following which they are exposed to polyplexes or polymer suspended in SFM. Cells were exposed to polyplexes or polymer/SFM (controls) for 600 s and exposed again to ECS for 400 s.

The current vs time trace files were exported and processed using Microsoft Excel and MATLAB. In all cases, initial current magnitudes less than -15 nA were required to indicate patching of sufficient quality for each ensemble of 20 cells. Data for ensembles with starting currents above -15 nA were not included in the analysis. The time averages of current from 4s prior to exposure (60 - 64 s from the beginning) was compared with the time averaged current 600-604 s (665 -669 s from the beginning) following exposure to polymers/polyplexes suspended in SFM. One way analysis of variance (ANOVA) followed by Tukey's multicomparison test was performed to determine the statistical significance of the difference in current changes across different treatments.

A



B

Phase	Main Channel	Traps	Traps and Compounds	Compounds
Preprime	1 psi (0-92 s) 0.3 psi (92-120 s)	5 psi (0-90 s) 1.5 psi (90-115 s)	6 psi (115-120s)	
Prime	1 psi (0-30 s) 0.4 psi (30-55 s)	6 Hg (50-55 s)	5 psi (0-20 s) 2 psi (20-50 s)	Not used
Trap	Pulse: 0 psi for 4.2s 0.25 psi for 0.8 s 15 such pulses	6 Hg (0-85 s)	Not used	Not used
Break	0.2 psi (0-20 s)	6 Hg (0-5 s) 10 Hg (5-15 s) 6 Hg (15-20 s)	Not used	Not used
Data Acquisition	0.16 psi (Duration of experiment)	6 Hg (Duration of experiment)	Not used	6 psi

C

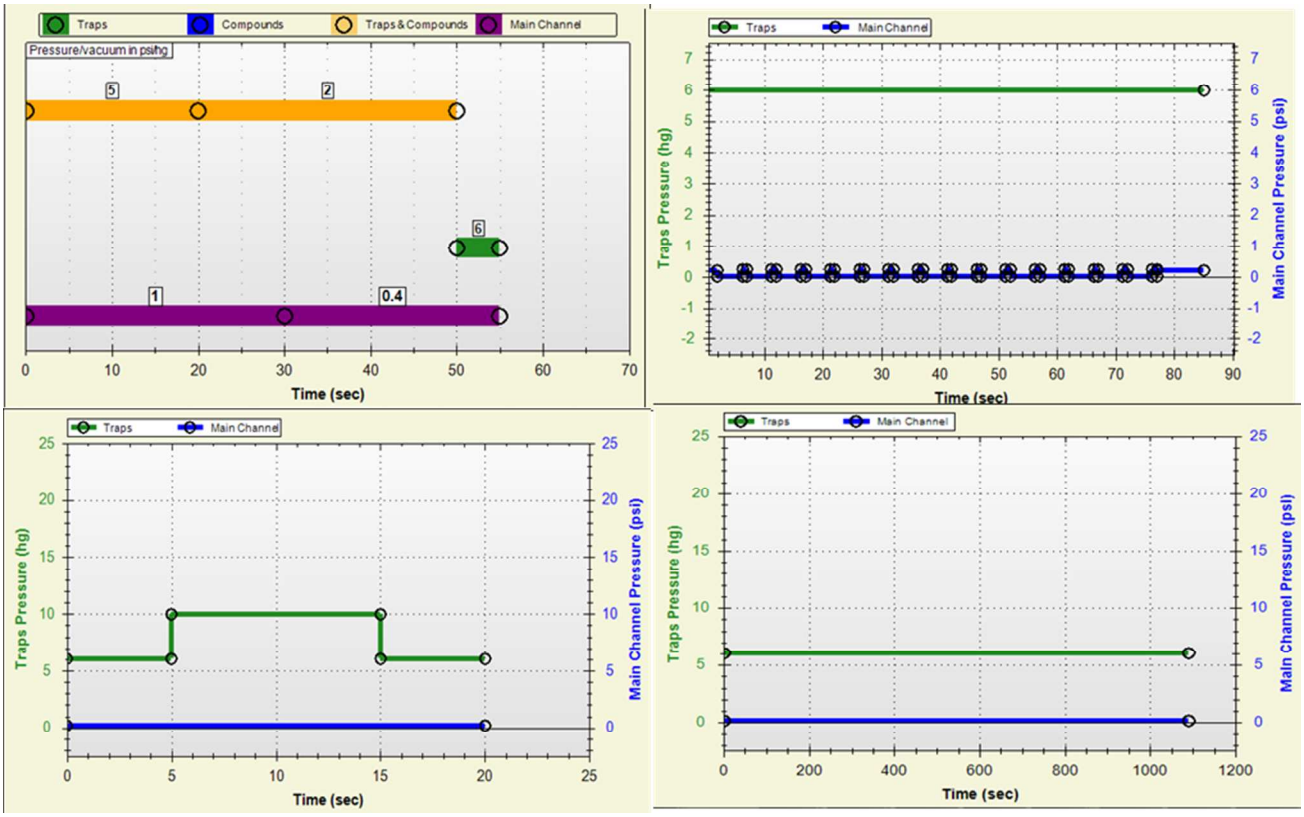
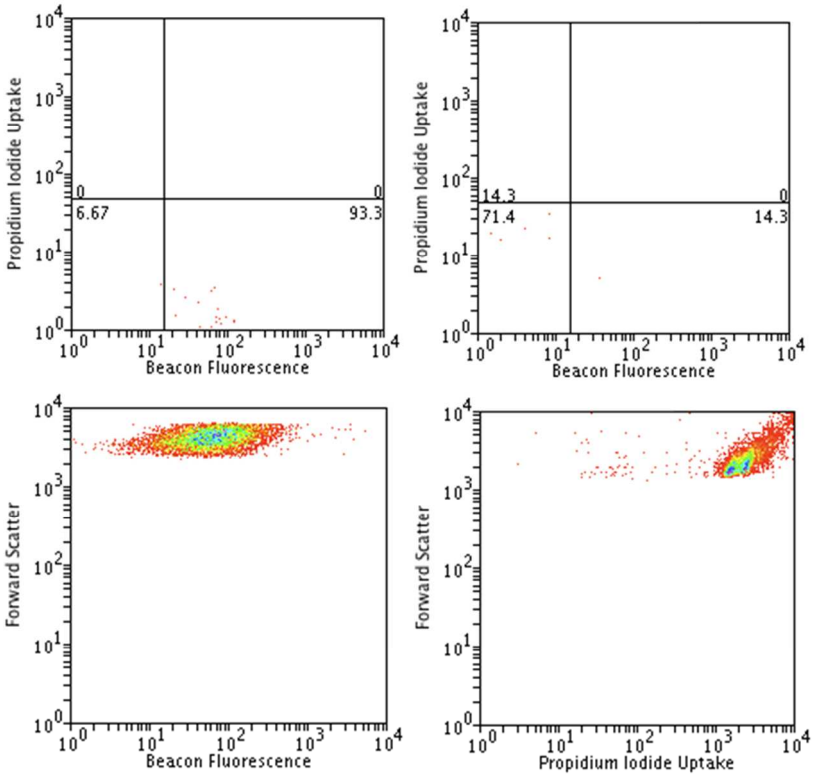


Figure S4. A shows the order and relative durations of the 4 phases in the experiment. B summarizes the pressure settings at the different phases. C is a graphic representation of the pressure settings.

Figure S5. Single Dye Controls Compensation was performed so as to have no cells in the third quadrant for the single dye control.

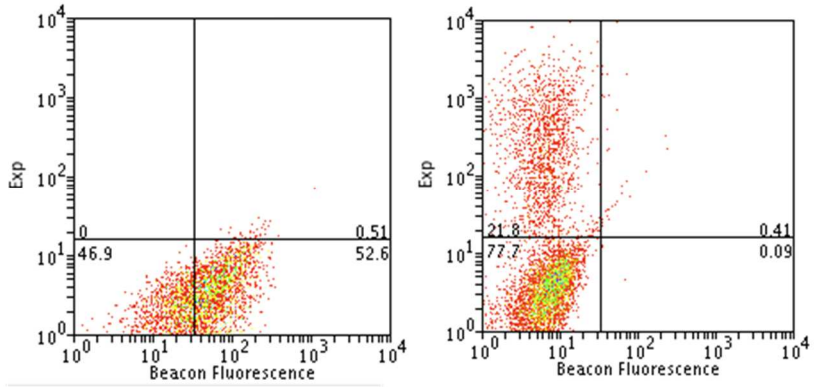
a) Single dye controls for Figure 1. Forward scatter vs. Beacon fluorescence plots are also presented to show normal cell population for MB only and Propidium Iodide only controls.

MB only control Propidium Iodide only control



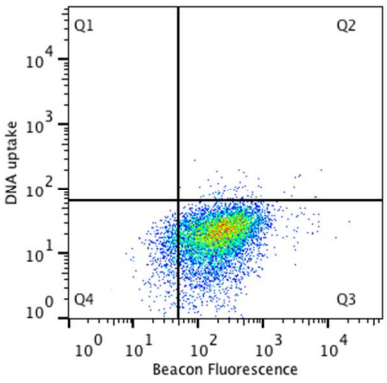
b) Single dye controls for Figure 3.

MB only control Expression only control



c) Single dye controls for Figure 4.

MB only control



DNA uptake only control

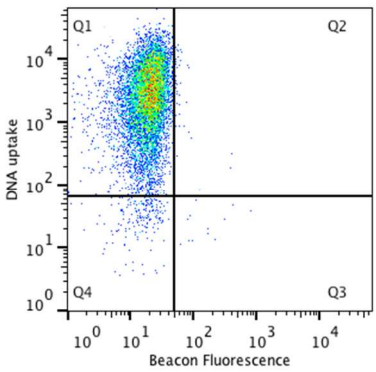
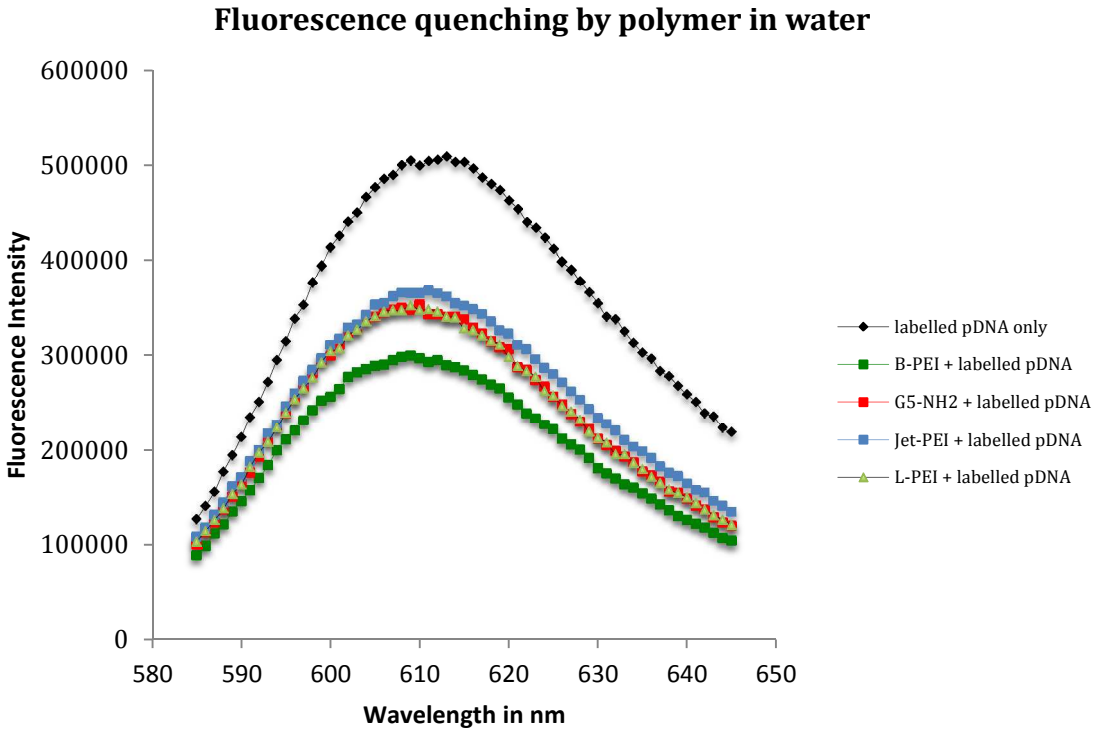
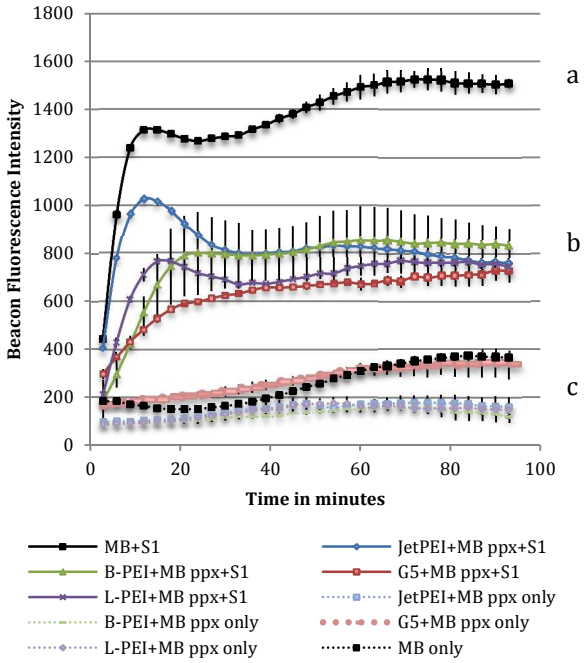


Figure S6 Fluorescence intensity quenching of rhodamine labeled DNA by B-PEI, G5, Jet-PEI and L-PEI in water.



In this study DNA was labeled with a fluorescent dye and it is possible that interaction with different polymers quench fluorescence to a different extent. To evaluate this possibility, the quenching capabilities of the polymers was studied (Figure S6). Polymer presence, causing polyplex formation, quenches dye emission but the polymers quench to a similar extent. The one exception is B-PEI, which gives 17% lower fluorescence than the other polymer/DNA samples. In Figure 1, the B-PEI polyplexes are observed to have greater fluorescence than the L-PEI or jetPEI samples. None of the conclusions reached are affected by this ~20% change in fluorescent emission.

Figure S7: Fluorescence of MB a) after treatment with S1 nuclease b) after complexing as a 10:1 N:P polyplex with L-PEI, B-PEI, G5 PAMAM, or jetPEI™ followed by S1 nuclease treatment c) controls of MB and polyplex with no S1 nuclease added.



(1) Spencer, C. I.; Li, N. Z.; Chen, Q.; Johnson, J.; Nevill, T.; Kammonen, J.; Ionescu-Zanetti, C.: Ion Channel Pharmacology Under Flow: Automation Via Well-Plate Microfluidics. *Assay and Drug Development Technologies* **2012**, *10*, 313-324.