

# **Trehalose-Based Block Copolycations Promote Polyplex Stabilization for Lyophilization and *in Vivo* pDNA Delivery**

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## Supplementary Information

### Materials

All reagents were obtained at the highest available purity from Thermo Fisher Scientific, Inc. (Pittsburgh, PA) or Sigma-Aldrich Co. LLC. (St. Louis, MO) and used as received unless noted otherwise. jetPEI was obtained from Polyplus-transfection SA (Illkirch, France). Glycofect was obtained from Techulon, Inc. via donation. 6-Methacrylamido-6-deoxy trehalose (MAT) was synthesized as previously described.<sup>1</sup> Propidium iodide, UltraPure™ Agarose-1000, trypsin, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), phosphate-buffered saline (PBS), Minimum Essential Medium with reduced serum (Opti-MEM), and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Life Technologies – Thermo Fisher Scientific (Carlsbad, CA). The pT2/CaL plasmid was prepared as previously described.<sup>2</sup> The Luciferase Assay Kit and cell lysis buffer were obtained from Promega Corporation (Madison, WI). Bio-Rad DC Protein Assay Reagent A, Reagent B and Reagent S were obtained from Bio-Rad Laboratories, Inc. (Hercules, CA). HepG2 and U87 cells were obtained from the American Type Cell Culture Collection (ATCC) (Manassas, VA). The cells were grown in complete DMEM [supplemented with 10% (v:v) fetal bovine serum and 1% antibiotic-antimycotic solution (containing penicillin, streptomycin, and amphotericin B)] at 37 °C and 5% CO<sub>2</sub> in a humidified incubator.

### Instrumentation

NMR spectra were recorded using a Bruker Avance III HD 500 MHz spectrometer in D<sub>2</sub>O purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). NMR data was analyzed using Bruker Top Spin version 3.1. UV-Vis data was collected with an Ocean Optics Inc. CUV 1 cm cuvette holder powered by a Mikropack DH-2000 Deuterium/Halogen open-close TTC lamp, and data was analyzed by Ocean Optics Inc. Basic Acquisition Software.

Size exclusion chromatography (SEC) was conducted using an Agilent 1260 High Performance Liquid Chromatograph running 1.0 wt% acetic acid/0.1 M Na<sub>2</sub>SO<sub>4</sub> as the eluent at a flow rate 0.4 mL/min on size exclusion chromatography columns [CATSEC1000 (7 μ, 50×4.6), CATSEC100 (5 μ, 250×4.6), CATSEC300 (5 μ, 250×4.6), and CATSEC1000 (7 μ, 250×4.6)] obtained from

Eprogen Inc. (Downers Grove, IL). Signals were acquired using Wyatt HELEOS II light scattering detector ( $\lambda = 662$  nm), and an Optilab rEX refractometer ( $\lambda = 658$  nm). SEC trace analysis was performed using Astra VI software (version 5.3.4.18), Wyatt Technologies (Santa Barbara, CA). Hydrodynamic diameter of the polyplexes was recorded via dynamic light scattering measurements (DLS) with a Malvern Zetasizer Nano ZA. MTT, protein, and luciferase assays were measured with a BioTek Synergy H1 plate reader (BioTek Instruments, Inc., Winooski, VT). Cy5-uptake was measured on a FACSVerse (Becton Dickinson Biosciences, San Jose, CA) flow cytometer. TEM images were obtained with a FEI Tecnai G2 Spirit BioTWIN (FEI, Hillsboro, OR) transmission electron microscope, operated at 120 kV.

Live animal and animal tissue imaging were performed on an IVIS Spectrum In Vivo Imaging system and data was analyzed with the Living Image software (PerkinElmer Inc., Waltham, MA). RT-qPCR was performed using an Eppendorf Mastercycler (software version 2.2; Eppendorf).

Statistical analysis was performed using JMP Pro Software (SAS Institute, Cary, NC) through the University of Minnesota Supercomputing Institute.

### Polymer Synthesis

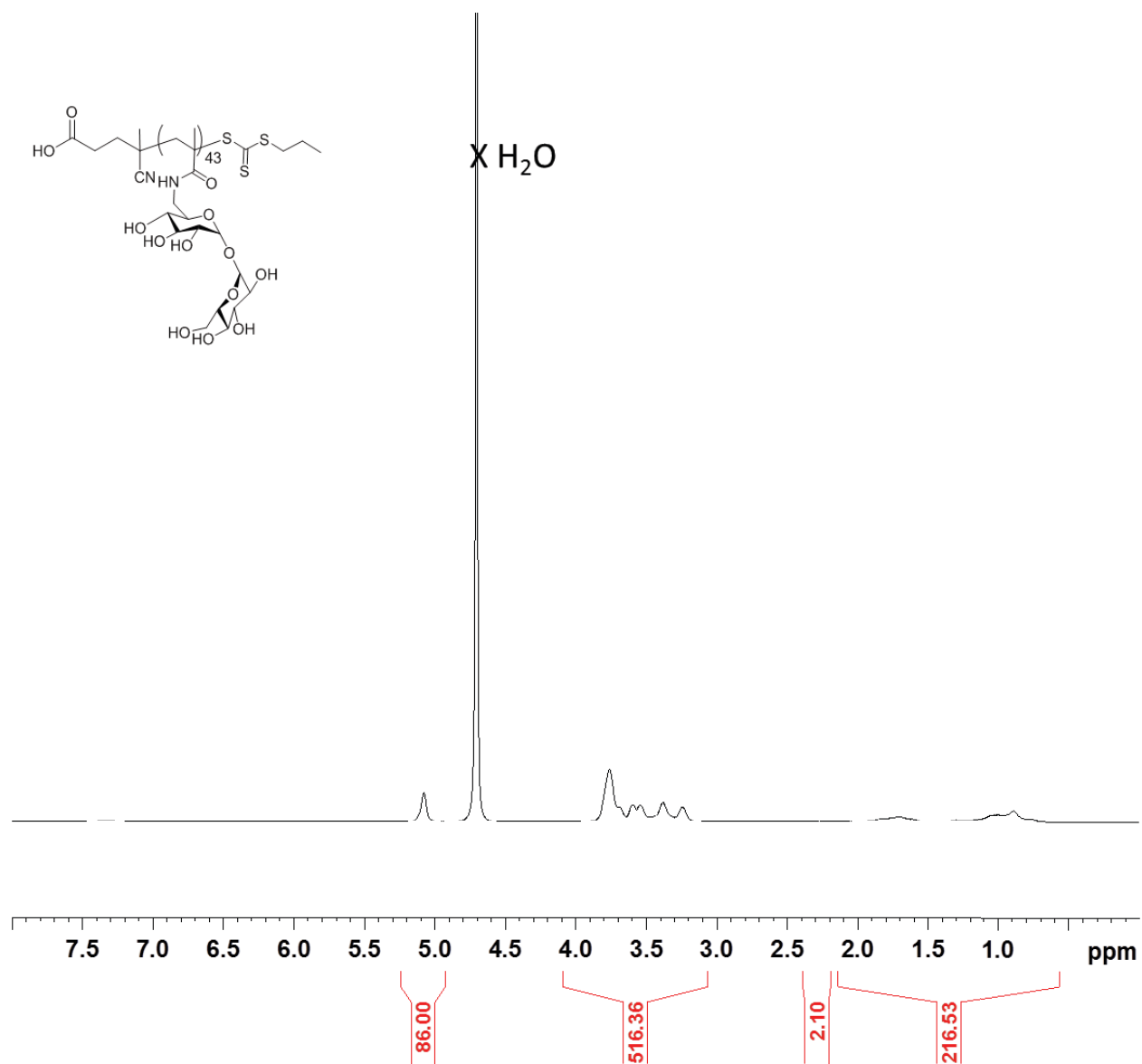
#### **Synthesis of poly(methacrylamidotrehalose) (pMAT<sub>43</sub>)**

pMAT 43 was synthesized as previously described.<sup>1</sup> Briefly, MAT (0.765 g, 1.867 mmol, 65 eq) was dissolved in 7.00 mL of 0.1 M acetate buffer (pH = 5.5). 4-cyano-4-(propylthiocarbonothioylthio)-pentanoic acid (CPP) (7.97 mg, 2.87x10<sup>-2</sup> mmol, 1 eq) was dissolved in 645  $\mu$ L of MeODH and added to the MAT solution, followed by 4,4'-azobis(4-cyanovaleric acid) (V-501) (0.805 mg, 2.87x10<sup>-3</sup> mmol, 0.1 eq) in 244  $\mu$ L of methanol (MeOH). Finally, 861  $\mu$ L of MeOD was added to a final volume of 1.75 mL of MeOH. Oxygen was removed by bubbling nitrogen for 45 min. The flask was heated to 70 °C for 6 h and the reaction was stopped by removing the septum and cooling the reaction mixture on ice. The polymer solution was dialyzed against ultra-pure water (3500 Da MWCO) acidified to pH 4-5 with HCl. Water changes were performed every 4 h. After 3 d of dialysis, the polymer solution was freeze

dried on a VirTis benchtopK lyophilizer at 62 mT with the condenser at  $-57.4^{\circ}\text{C}$  lyophilized to yield 454 mg of white solid.

SEC (eluent: 1.0 wt% acetic acid/0.1 M  $\text{Na}_2\text{SO}_4$ ). A flow rate of 0.4 mL/min, light scattering detector ( $\lambda = 662$  nm) and refractometer ( $\lambda = 658$  nm).  $M_n=17.7$  kDa, degree of polymerization (n) =42.5~43, PDI = 1.06.

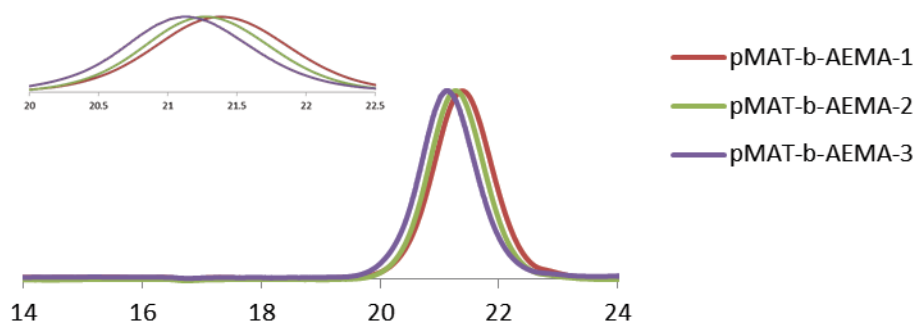
$^1\text{H-NMR}$  (500 MHz,  $\text{D}_2\text{O}$ )  $\delta$  ppm: 5.26 – 4.96 (bs, 86H), 4.00 – 3.04 (m, 516H), 2.35 – 2.21 (bs, 2H), 1.79 – 0.61 (m, 217H).



**Figure S1.**  $^1\text{H}$  NMR spectrum of pMAT<sub>43</sub>.

### Synthesis of cationic diblock copolymers pMAT-b-AEMA 1-3

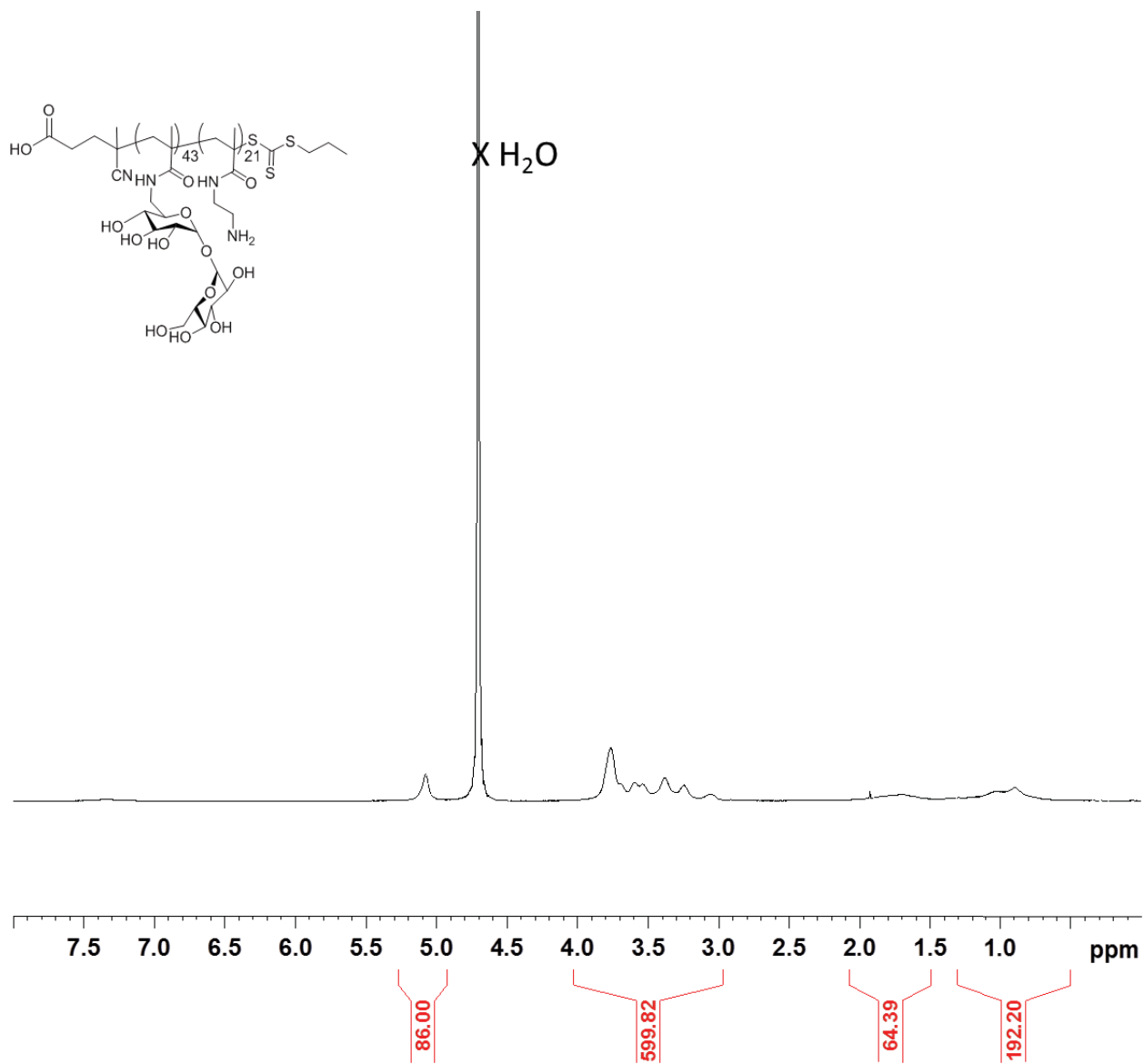
Cationic diblock copolymers were synthesized as previously reported.<sup>1</sup> Briefly, pMAT<sub>43</sub> (327 mg,  $1.56 \times 10^{-2}$  mmol, 1.00 eq) was dissolved in 3.00 mL of 1.0 M acetate buffer (pH = 5.5) and added to a schlenk tube containing aminoethylmethacrylamide hydrochloride (AEMA · HCl, 311 mg, 1.89 mmol, 121 eq). V-501 (0.44 mg,  $1.56 \times 10^{-3}$  mmol, 0.10 eq) was dissolved in 0.78 ml of 1.0 M acetate buffer (pH = 5.5) and added to the schlenk tube prior to sealing the tube. Deoxygenation was achieved via bubbling nitrogen gas for 45 min. The tube was heated to 70 °C. Aliquots (1.25 mL) were removed via syringe at 30 min (pMAT-b-AEMA-1) and 60 min (pMAT-b-AEMA-2). Each aliquot was exposed to air and cooled in liquid nitrogen to stop polymerization. After 90 min (pMAT-b-AEMA-3), the reaction was halted by septum removal and submerging the schlenk tube in liquid nitrogen. All three copolymers were dialyzed (3500 Da MWCO) against 3x4L of 0.5 M NaCl solution, followed by 3x4L 0.1 M NaCl and finally 6x4L of ultra-pure water. All dialysis media were acidified with HCl to pH 4-5. Polymer solutions were lyophilized as described above to yield white, flocculent powders. All experiments were completed using polymers pMAT-b-AEMA-1, -2, and -3.



**Figure S2.** GPC traces of pMAT-b-AEMA series of polymer. Lower retention time indicates higher molecular weight.

MAT<sub>43</sub>-*b*-AEMA<sub>21</sub>(**pMAT-b-AEMA-1**), SEC (eluent: 1.0 wt% acetic acid/0.1 M Na<sub>2</sub>SO<sub>4</sub>). A flow rate of 0.4 mL/min, light scattering detector ( $\lambda = 662$  nm) and refractometer ( $\lambda = 658$  nm). Mn=20.4 kDa, PDI=1.07.

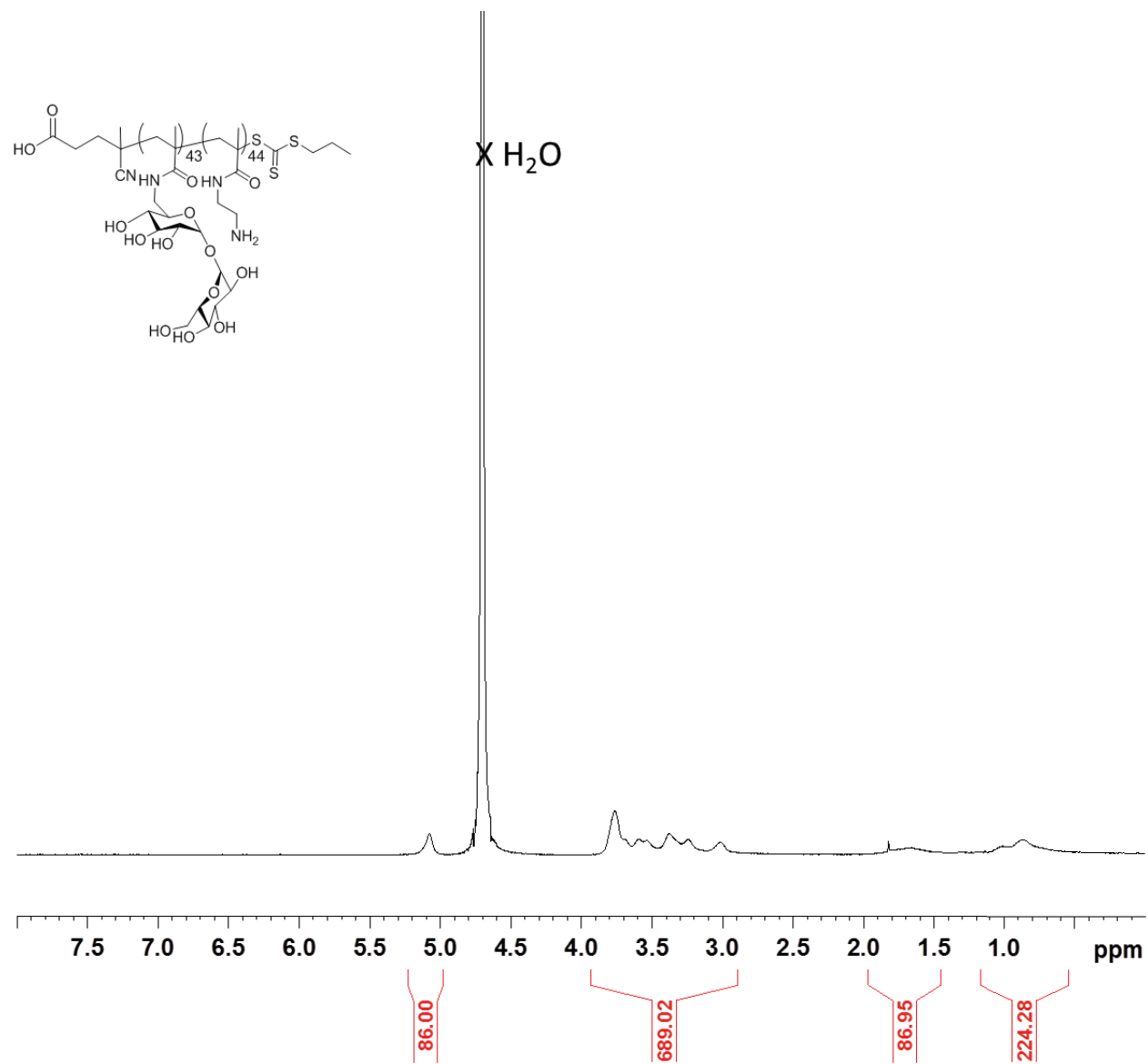
<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  ppm: 5.18 – 4.97 (bs, 86H), 3.94 – 2.94 (m, 600H), 2.03-1.49 (m, 64H), 1.16 – 0.66 (m, 192H).



**Figure S3.** <sup>1</sup>H NMR spectrum of **pMAT-b-AEMA-1**.

MAT<sub>43</sub>-*b*-AEMA<sub>44</sub>(**pMAT-b-AEMA-2**), SEC (eluent: 1.0 wt% acetic acid/0.1 M Na<sub>2</sub>SO<sub>4</sub>). A flow rate of 0.4 mL/min, light scattering detector ( $\lambda = 662$  nm) and refractometer ( $\lambda = 658$  nm). Mn=23.3 kDa, PDI=1.07.

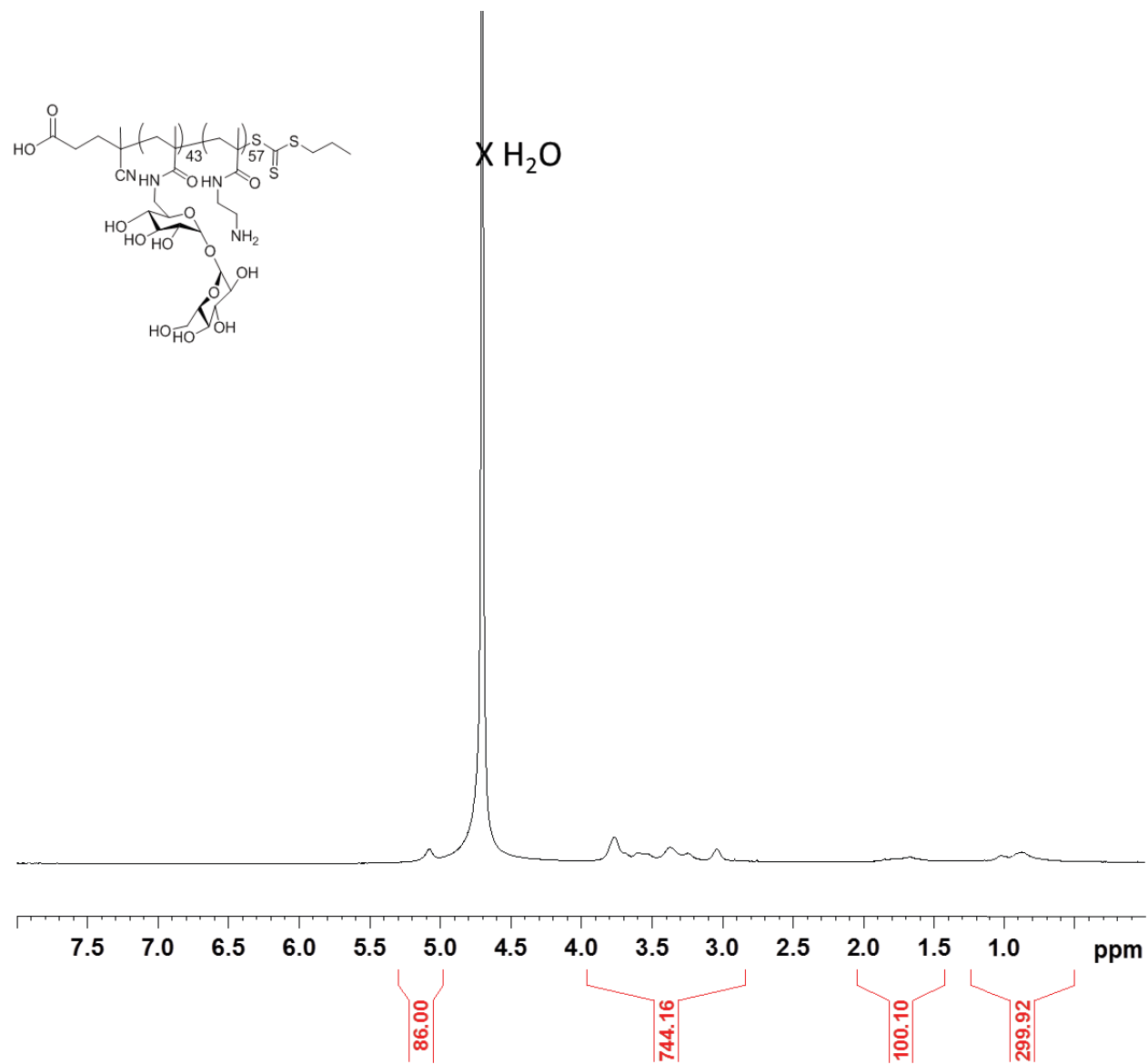
<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  ppm: 5.18 – 4.97 (bs, 86H), 3.94 – 2.94 (m, 689H), 2.03-1.49 (m, 87H), 1.16 – 0.66 (m, 224H).



**Figure S4.** <sup>1</sup>H NMR spectrum of **pMAT-b-AEMA-2**.

MAT<sub>43</sub>-*b*-AEMA<sub>57</sub>(**pMAT-b-AEMA-3**), SEC (eluent: 1.0 wt% acetic acid/0.1 M Na<sub>2</sub>SO<sub>4</sub>). A flow rate of 0.4 mL/min, light scattering detector ( $\lambda = 662$  nm) and refractometer ( $\lambda = 658$  nm). Mn=25.0 kDa, PDI=1.08.

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  ppm: 5.18 – 4.97 (bs, 86H), 3.94 – 2.94 (m, 744H), 2.03-1.49 (m, 100H), 1.16 – 0.66 (m, 300H).



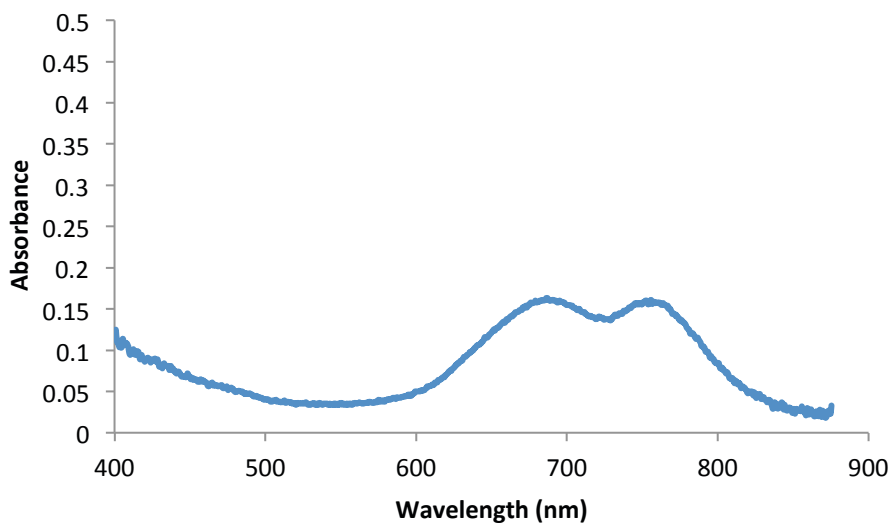
**Figure S5.** <sup>1</sup>H NMR spectrum of **pMAT-b-AEMA-3**.



## Synthesis of Cy7-pMAT-b-AEMA-2

The polymer was labeled using an NHS-Cy7 fluorophore. **pMAT-co-AEMA-2** (10.0 mg,  $4.30 \times 10^{-4}$  mmol, 0.0190 mmol primary amine) was dissolved in 900  $\mu\text{L}$  of  $\text{H}_2\text{O}$ . Cy7 functionalized with N-hydroxysuccinamide (NHS-Cy7) in DMF (26  $\mu\text{L}$ , 1.0 mg/ 10  $\mu\text{L}$ , 0.00038 mmol) was added followed by 97.4  $\mu\text{L}$  of DMF to target 1 fluorophore/50 amine residues. The mixture was vortexed for 30 s and allowed to proceed for 4 h in the dark. The labeled polymer was purified via dialysis (3500 MWCO) against  $\text{H}_2\text{O}$  (acidified to pH  $\sim 5.5$  with HCl) for 2 days and lyophilized using the same conditions listed before to yield a blue flocculent solid.

Extent of labeling was quantified measuring the absorbance of a 0.2 mg/ mL of labeled polymer in  $\text{H}_2\text{O}$  at 750 nm via UV-Vis ( $\epsilon = 199000$  at 750 nm, Abs = 0.16, Figure S2) giving approximately 1 Cy7 fluorophore / 10 polymer chains.



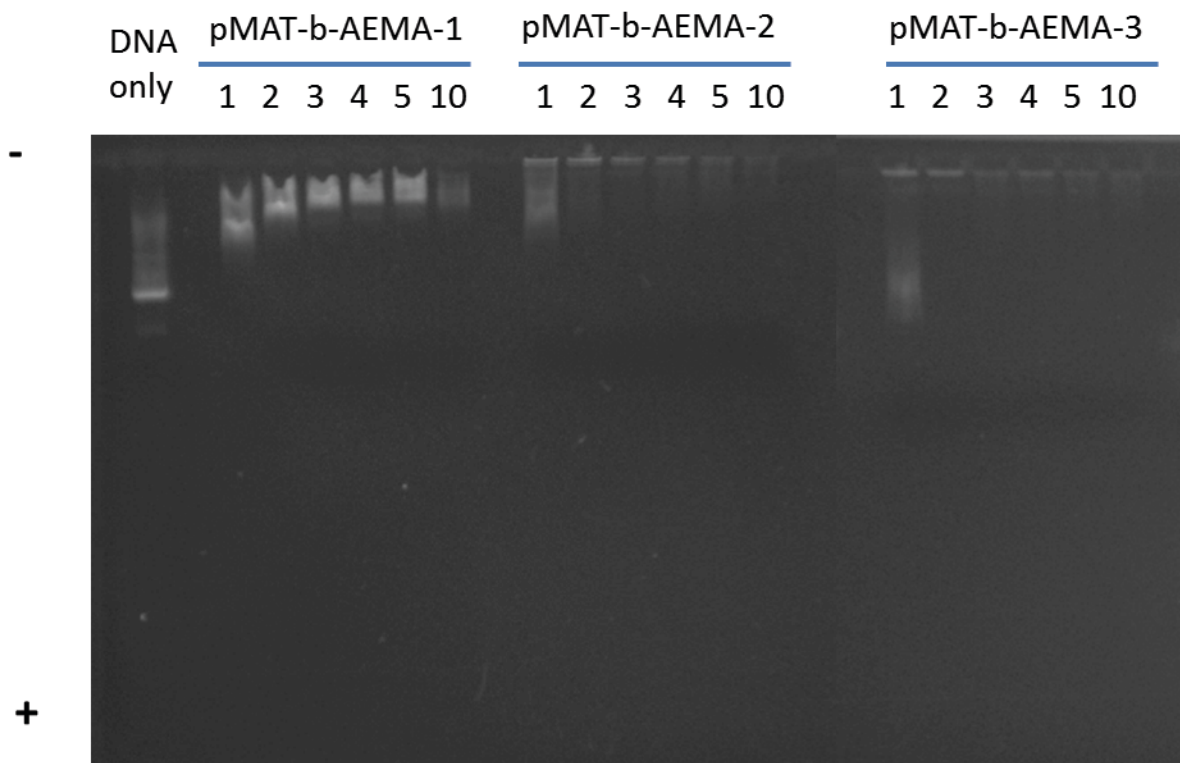
**Figure S6.** Absorbance of **Cy7-pMAT-b-AEMA-2** in  $\text{H}_2\text{O}$  used to calculate labeling efficiency ( $\sim 1$  Cy7 per 10 polymer chains) from the extinction coefficient for NHS-Cy7 (provided by the manufacturer, Lumiprobe Corp.).

## Polyplex Formation For Gel Electrophoresis and Tissue Culture (In Vitro) experiments

Polymer solution at an appropriate concentration in water was added to a 400  $\mu\text{M}$  solution of pDNA in DNase/RNase-free distilled water in an equal volume to yield polyplexes of (nitrogen to phosphate ratios) N/P = 7, 14, and 21 at a final concentration of 200  $\mu\text{M}$ . The nitrogen ratios were calculated based on the concentrations of amines (either pendant from AEMA blocks or within the backbone of jetPEI or Glycofect). The solutions were incubated at 23  $^{\circ}\text{C}$  for 1 h prior to further use.

### Gel Electrophoresis

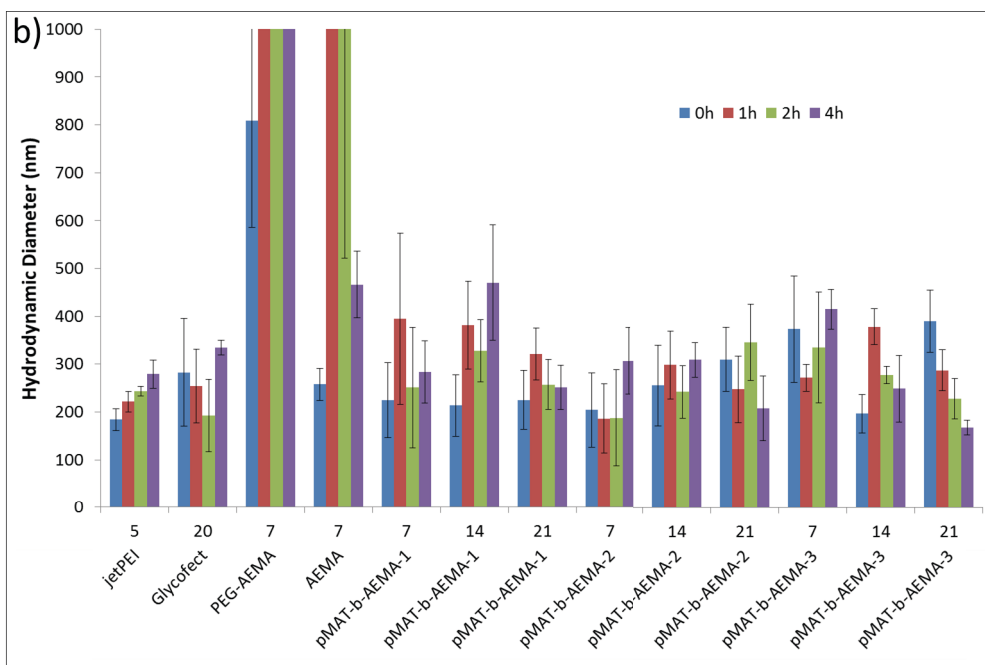
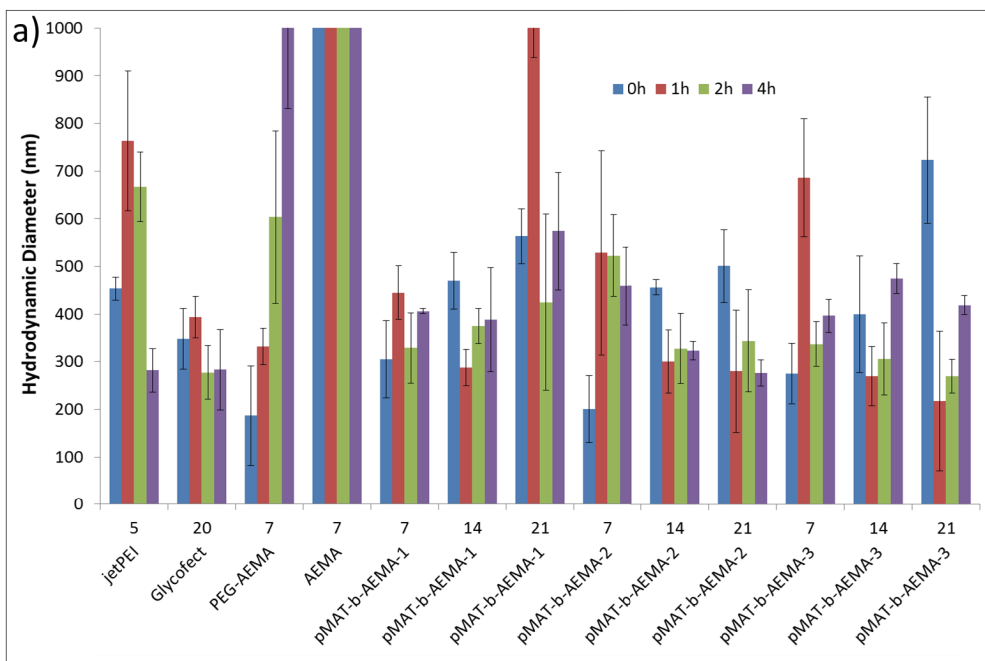
A gel electrophoresis mobility shift assay was run to determine the minimal amount of polymer needed to achieve complete binding of the pDNA. 10  $\mu\text{L}$  of each polyplex solution formulated at various N/P ratios were formulated and diluted with 10  $\mu\text{L}$  of water to achieve a concentration of 100  $\mu\text{M}$  and were incubated at 25  $^{\circ}\text{C}$  for 1 h to allow polymer-pDNA binding. The polyplex suspensions were run on agarose gels (0.6%) containing 6  $\mu\text{g}$  ethidium bromide at 60 volts for 80 min. Images were obtained using 312 nm UV light.



**Figure S7.** Gel electrophoresis mobility shift assay of polyplexes at various N/P. All three polymers show complete binding of DNA by N/P=3.

#### Dynamic Light Scattering (DLS)

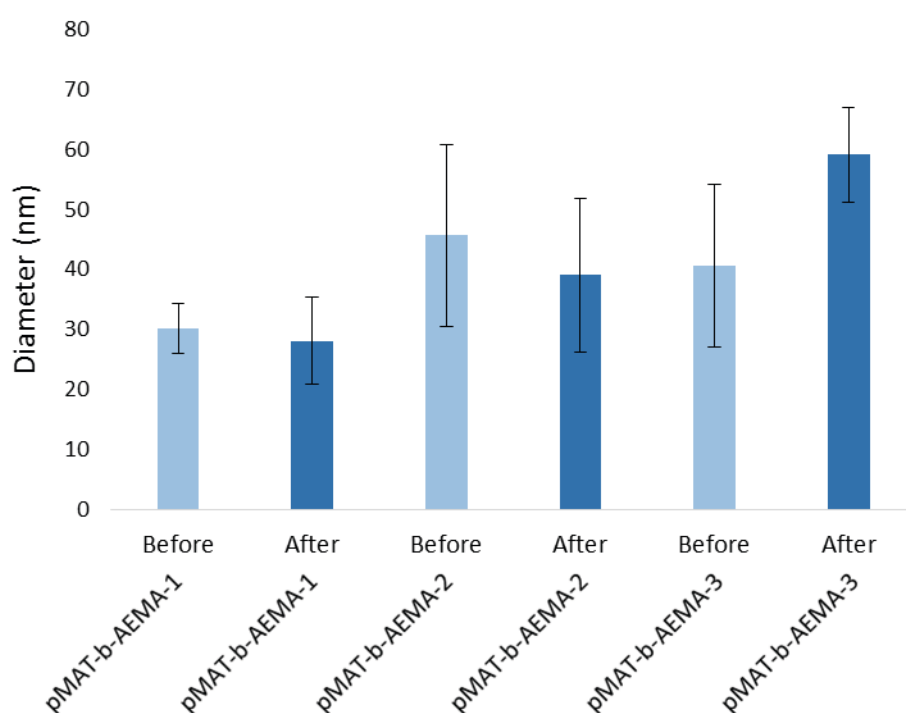
DLS experiments were run in DMEM containing 10% FBS. Briefly, polyplexes were formulated at N/P 7, 14, and 21 in H<sub>2</sub>O and incubated for 1 h at a concentration of 200  $\mu$ M in pDNA (to allow complexation) before being diluted with DMEM containing 10% FBS by volume. For DLS studies prior to lyophilization, the polyplexes were diluted to a final concentration of 100  $\mu$ M (T = 0 h). For the post-lyophilization DLS, polyplexes were prepared and lyophilized as previously described, reconstituted with water for 1 hour, then diluted with DMEM with 10% FBS to 133  $\mu$ M. Size measurements were taken at 25 °C using a 173° detection angle at times of 0 h, 1 h, 2 h, and 4 h.



**Figure S8.** Polyplex hydrodynamic diameters determined by DLS in DMEM plus 10% FBS after a) one round of lyophilization then b) a second round of lyophilization. Each measurement is the average of three data points with error bars representing the standard deviation.

## Transmission Electron Microscopy (TEM) Imaging

3  $\mu\text{L}$  of polyplex solution (formulated with pDNA and polymers **pMAT-b-AEMA-1**, **-2**, or **-3**) prepared at N/P=10 were applied to a 300-mesh carbon coated copper grid (Ted Pella, Inc., Redding, CA). Excess solution was removed with filter paper after 60 s. Negative stain (3.0  $\mu\text{L}$  1% uranyl acetate solution) was applied in triplicate to the sample to facilitate visualization. Images were recorded using Eagle<sup>TM</sup> 2k CCD camera, and phase contrast was enhanced at 10 to 12  $\mu\text{m}$  underfocus. Images were saved as TIFF files and analyzed by counting pixels in Microsoft Paint. Image S9 below summarizes the image analyses.



**Figure S9.** Polyplex diameter as measured by analysis of TEM images. Each data point is the average of at least eight polyplexes in each sample, and the standard deviation is shown by the error bar.

## Lyophilization

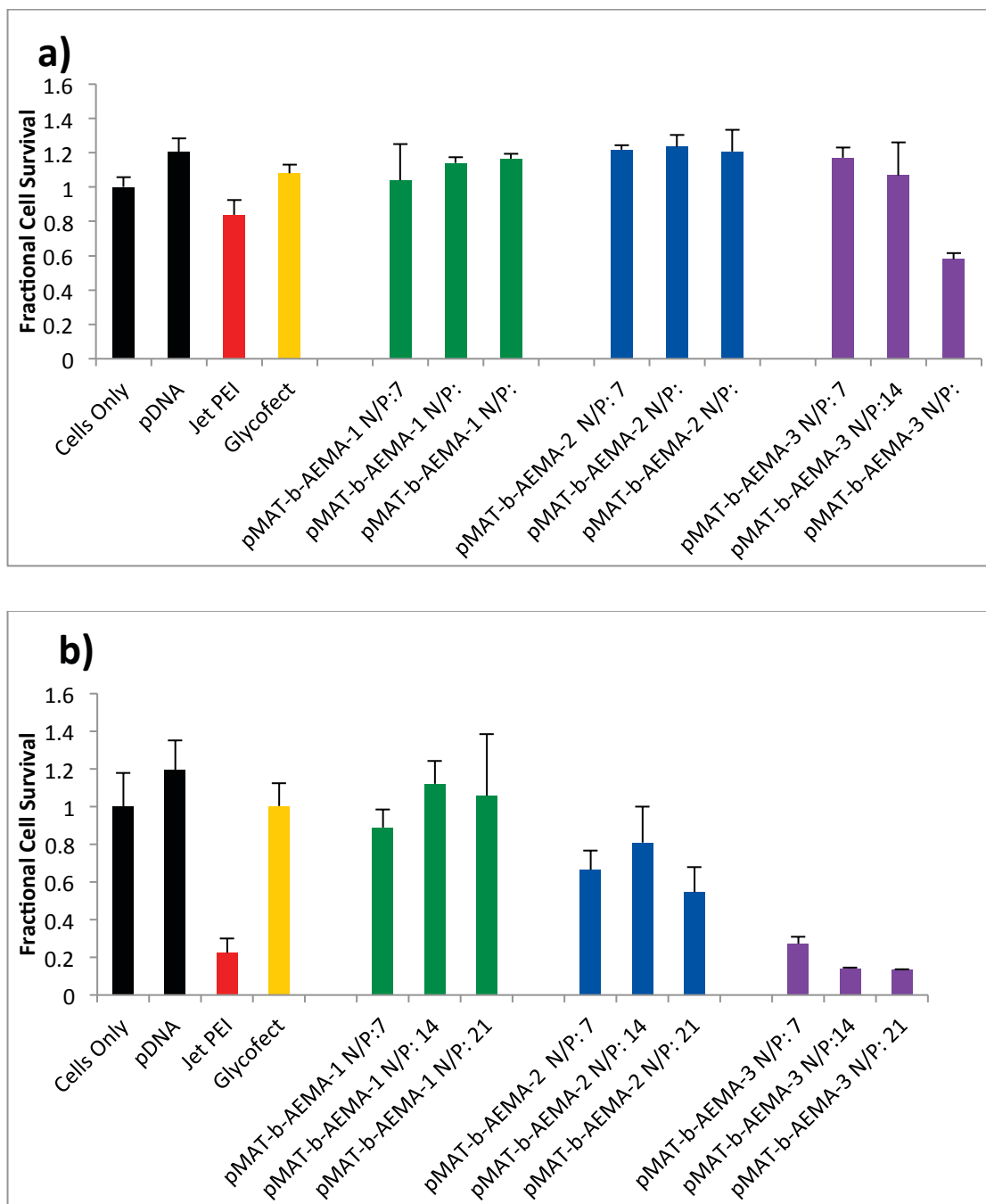
For studies involving lyophilization, polyplexes were formulated following the previously described procedure at a final concentration of 200  $\mu\text{M}$  in  $\text{H}_2\text{O}$ . The polyplexes were incubated at 23  $^\circ\text{C}$  for 1 h then frozen in liquid nitrogen and freeze dried on a VirTis benchtopK lyophilizer

at 62 mT with a condenser at  $-57.4^{\circ}\text{C}$ . The lyophilized powder was reconstituted to  $200\ \mu\text{M}$  in  $\text{H}_2\text{O}$  and allowed to incubate at  $23\ ^{\circ}\text{C}$  for 1 h before proceeding with the relevant assay.

## **Cell Culture Experiments**

### MTT Assay

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was used to estimate the cytotoxicity of the polyplexes. MTT is reduced to purple formazan in living cells via mitochondrial reductase. HepG2 or U87 cells were seeded at 50,000 cells/well in 24-well plates 24 h prior to transfection. Polyplexes were formed following the previously described procedure. Polyplexes were formulated as described above and transfection media ( $200\ \mu\text{L}$  of either Opti-MEM or DMEM containing 10% FBS) was added to each well; 4 h later, complete DMEM containing 10% FBS (1 ml) was added to each well. Following 24 h, the media was aspirated and cells were washed with PBS ( $500\ \mu\text{L}/\text{well}$ ). 10% FBS supplemented DMEM (1 mL) with  $0.5\ \text{mg}/\text{mL}$  of MTT was added to each well and incubated for 1 h. The media was then replaced with  $600\ \mu\text{L}$  of DMSO for 15 min at  $23\ ^{\circ}\text{C}$  to promote cell lysis.  $200\ \mu\text{L}$  aliquots were transferred to a well of a 96-well plate for analysis by colorimeter with wavelength at 570 nm. Non-transfected cells were used for normalizing the data.

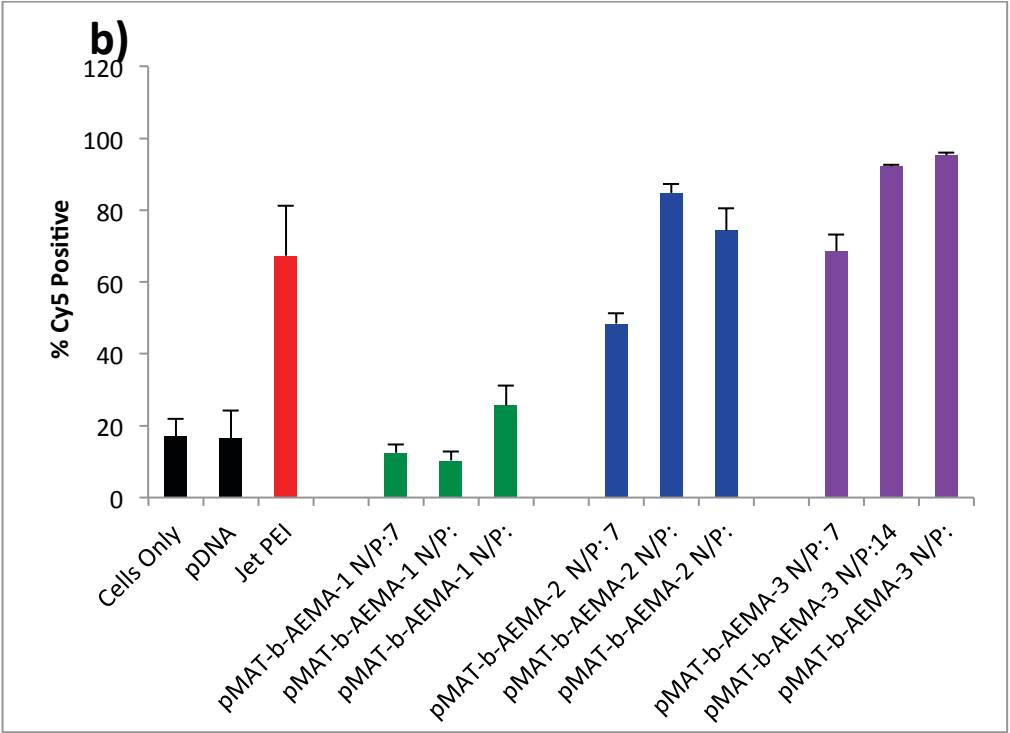
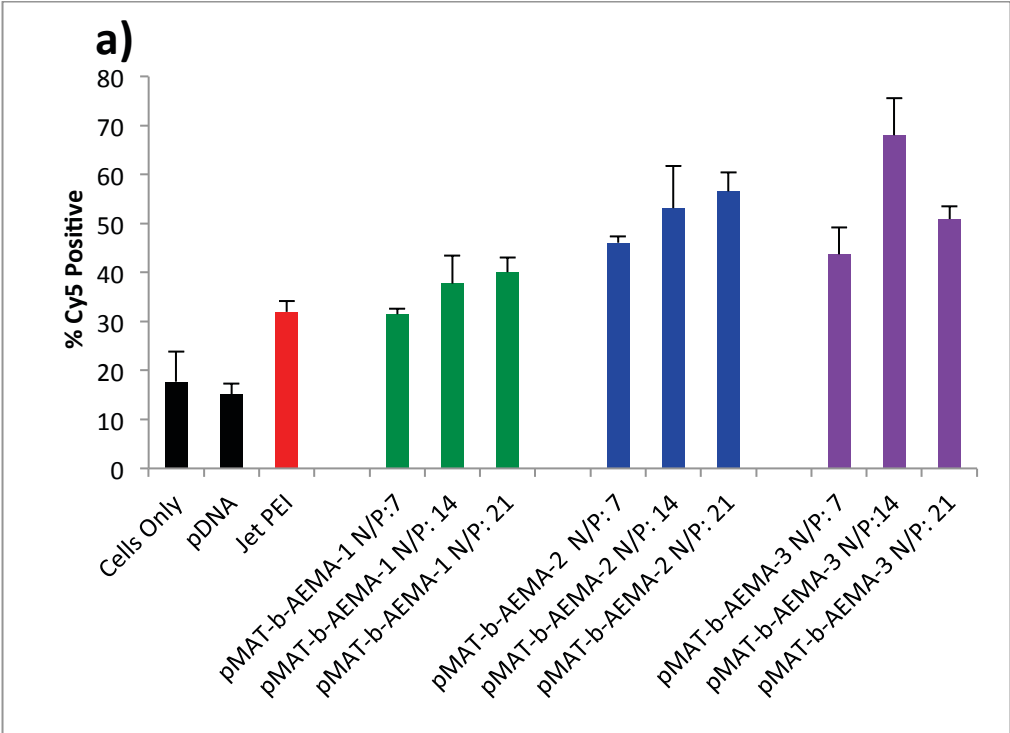


**Figure S10.** Cell survival following incubation of cells with **pMAT-b-AEMA-1**, **-2**, and **-3** polyplexes for 48 h in Opti-MEM with a) HepG2 cells and b) U87 cells at N/P ratios of 7, 14, 21. All results are normalized to a control sample containing cells that underwent no treatment and were allowed to proliferate normally for 48 hours in Opti-MEM (Cells Only). Bars represent the average of three separate experiments and error bars represent the standard deviation.

### Cellular Uptake Assay

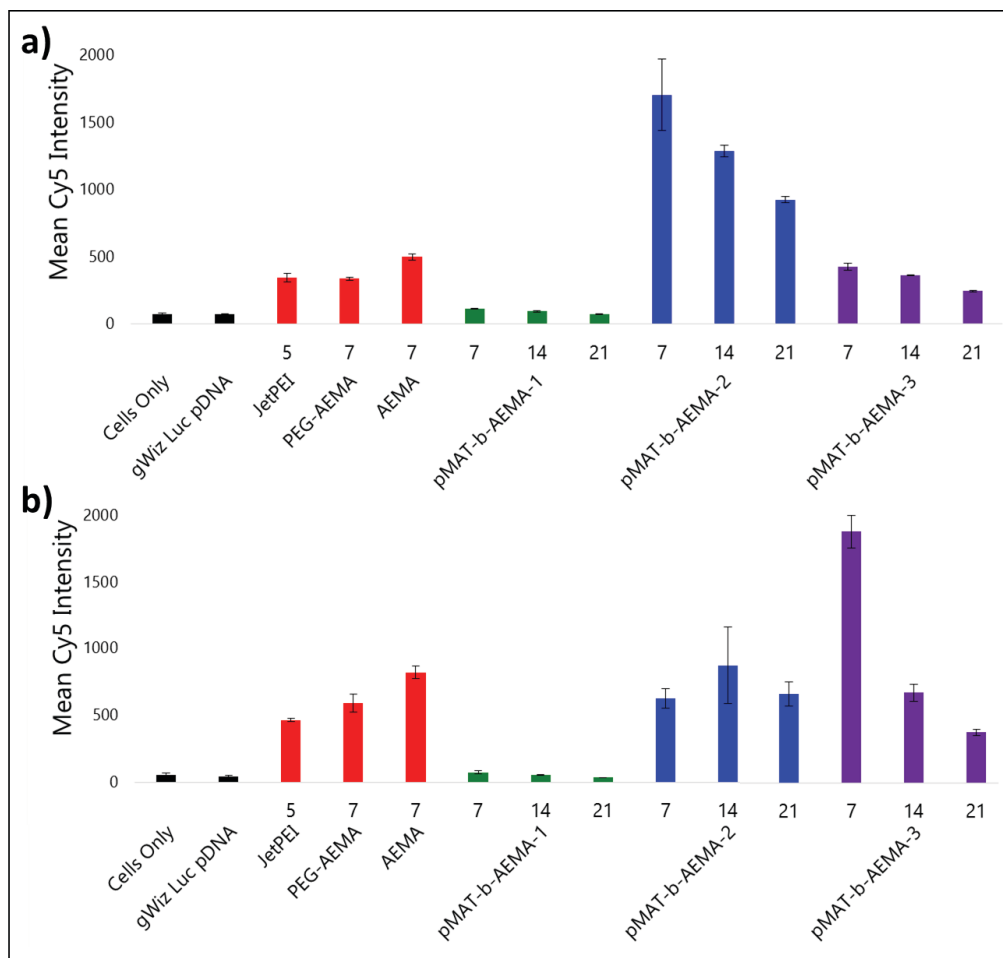
Flow cytometry was performed to examine the cellular uptake of Cy5-labeled pDNA 4 h post-transfection. HepG2 or U87 cells were seeded at 300,000 cells/well in 6-well plates 24 h prior to transfection. To transfect the cells, 66  $\mu\text{L}$  of polyplex solution were prepared following the previously described protocol above. Each polyplex solution was added into 1,584  $\mu\text{L}$  of pre-warmed (36  $^{\circ}\text{C}$ ) either Opti-MEM or DMEM containing 10% FBS; 500  $\mu\text{L}$  was added to each well. After 4h, the media was removed and cells were exposed to trypsin (0.05% (w/v), 500  $\mu\text{L}$ /well) for 3 min to detach the cells from the plate, then complete DMEM (500  $\mu\text{L}$ /well) was added to inhibit further digestion. Cell suspensions were collected and centrifuged at 1000 rpm for 10 min at 4  $^{\circ}\text{C}$  (Eppendorf 5810R centrifuge). The supernatant was removed and cells were washed twice with 0.5 mL PBS and centrifuged to remove any non-internalized polyplexes. Finally, 1 mL PBS was added and the cellular suspensions were kept on ice prior to flow cytometer analysis. Propidium iodide (1.0 mg/mL, 2.5  $\mu\text{L}$ ) was added prior to analysis. Twenty thousand events were counted for each sample. The threshold fluorescence level was defined by manually adjusting the positive region such that <1% of negative control cells were positive for fluorescence. Each experiment was performed in triplicate.





**Figure S11.** Cellular uptake of **pMAT-b-AEMA-1**, **-2**, and **-3** polyplexes formed with Cy5-labeled pDNA at N/P ratios of 7, 14, and 21 and incubated with cells for 4 h in Opti-MEM in a)

HepG2 and b) U87 cells. Bars represent the average of three separate experiments and error bars represent the standard deviation.



**Figure S12.** Cellular uptake plotted by mean fluorescence intensity of **pMAT-b-AEMA-1, -2,** and **-3** polyplexes formed with Cy5-labeled pDNA at N/P ratios of 7, 14, and 21 and incubated with cells for 4 h in DMEM in a) HepG2 and b) U87 cells. Bars represent the average of three separate experiments and error bars represent the standard deviation.

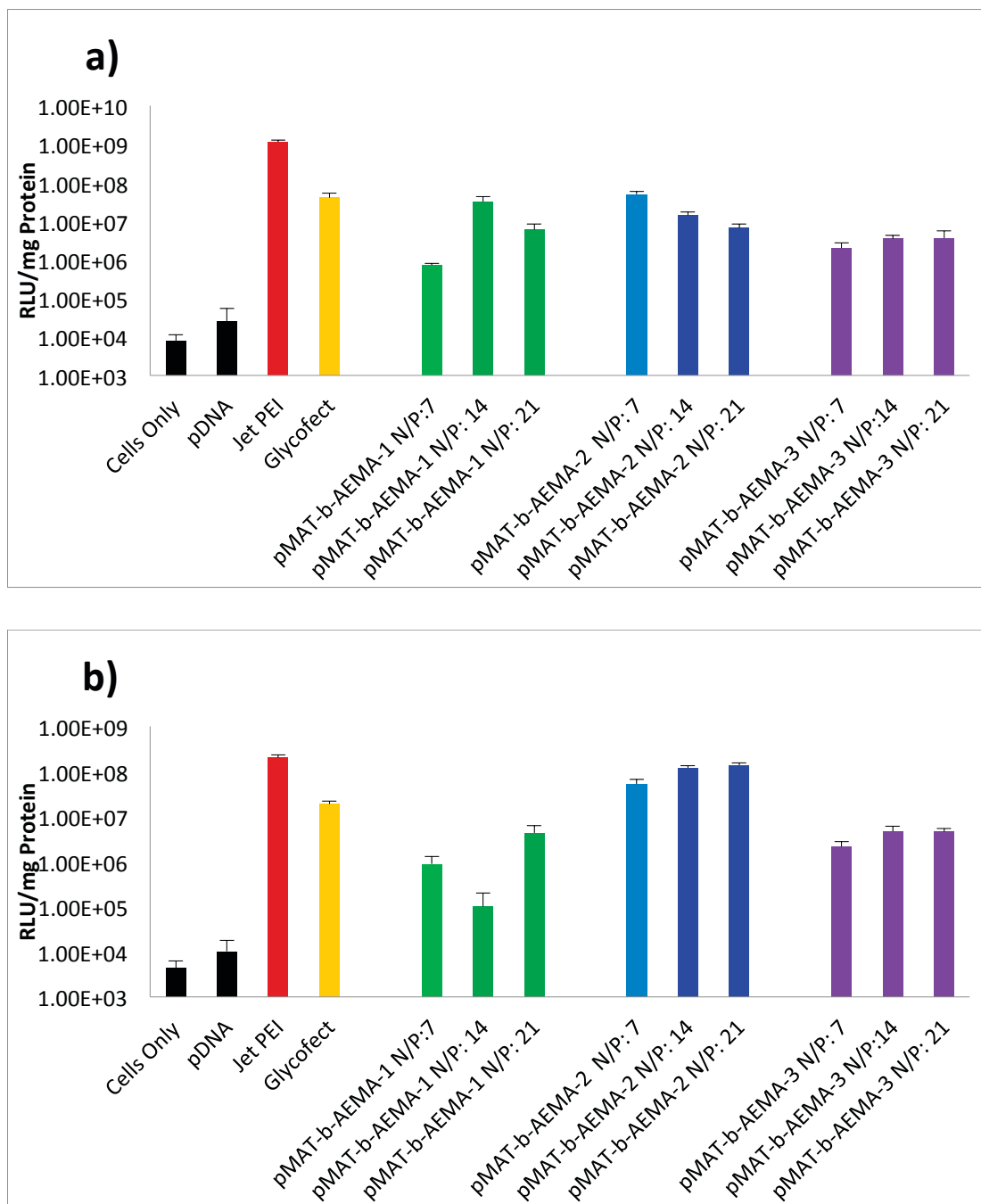
### Luciferase Gene Expression

Cells (HepG2 and U-87) were seeded at 50,000 cells/well in 24-well plates 24 h prior to transfection. Polyplexes were prepared following the previously described procedure described above. Polyplex solutions were diluted with pre-warmed (37 °C) Opti-MEM or DMEM with

10% FBS. Cells were washed with PBS before the addition of 200  $\mu$ L of the transfection solution.

Cells were incubated with polyplex solutions for 4 h before complete DMEM (1 mL) was added. After 48 h, the cells were washed with 500  $\mu$ L of PBS and treated with 1x cell lysis buffer for 15 min at 23 °C. Aliquots (5  $\mu$ L) of cell lysate were examined on 96-well plates with a luminometer for luciferase activity with a 10 s scan with 100  $\mu$ L of luciferase substrate added in each well immediately prior to relative light unit (RLU) determination. The average of duplicate fluorescence measurements was utilized for each sample calculation and each experiment was performed in triplicate.

The amount of protein (mg) in cell lysates was calculated using a standard curve generated with bovine serum albumin by following the protocol included in Bio-Rad DC protein assay kit. The relative light unit (RLU/mg) protein was then calculated and averaged across replicate wells for comparison. The protein and luciferase levels of non-transfected cells were used for normalizing the data and each experiment was performed in triplicate.



**Figure S13.** Luminescence of cell lysate following addition of luciferin in a) HepG2 cells and b) U87 cells. Cells were incubated with **pMAT-b-AEMA** polyplexes for 4 h in Opti-MEM followed by 48 h in full cell growth medium. Bars represent the average of three separate experiments and error bars represent the standard deviation.

### Tail Vein Injections

Wild-type (WT) C57BL/6J 6wk-old mice were purchased from Jackson Laboratories (Sacramento, CA). All mice were maintained under AAALAC-accredited (Association for Assessment and Accreditation of Laboratory Animal Care) specific pathogen-free conditions. Appropriate amounts of polyplexes (10 µg or 25 µg of pDNA/dose at desired N/P of polymer) were prepared in a 5% by weight aqueous dextrose solution (D5W, Hospira, Inc.), and a 200 µL dose of polyplexes was injected through the tail vein of experimental animals. After injection, animals were returned to their colony. Mice were imaged for transgene expression about 24 and 48 h after injection of the DNA solution.

### Hydrodynamic Injections

Wild-type (WT) C57BL/6J 6wk-old mice were purchased from Jackson Laboratories. All mice were maintained under AAALAC-accredited, specific pathogen-free conditions. Appropriate amounts of plasmid DNA were prepared in D5W solution and injected through the tail vein of experimental animals using a hydrodynamics-based procedure as previously described.<sup>3</sup> Briefly, animals to be injected were weighed. A DNA solution volume equivalent to 10% of the weight of the mouse was calculated. Animals were anesthetized with a ketamine cocktail as per protocol. All animals were injected with the appropriate DNA solution in 3 to 4 s. After injection, animals were placed on a heating pad to recover and returned to their colony. Mice were imaged for transgene expression about 24 and 48 h after injection of the DNA solution.

### Bioluminescence Imaging *In Vivo*

100 µL of luciferin substrate solution (28.5 mg/mL) was injected intraperitoneally to each mouse. 3 to 5 min later, mice were imaged for 1 min using a Xenogen Spectrum CCD camera system (Xenogen, Alameda, CA) according to the manufacturer's instructions. Each experiment was performed in triplicate.

### Euthanizing and Tissue Collection/Processing

Mice were euthanized by CO<sub>2</sub> (carbon dioxide) inhalation, perfused with saline, and selected organs were resected and preserved for analyses. Plasma and tissues were stored at -80 °C. Frozen liver tissues were homogenized by mortar and pestle, and a 100 mg tissue sample was

removed for DNA processing. All other tissues were resected from the body and placed in DNA extraction solution directly. DNA extraction solution was comprised of Qiagen Cell Lysis buffer (Qiagen, Germantown, MD) and Proteinase K (20 mg/mL; Fisher Scientific, Fair Lawn, NJ). 700  $\mu$ L of Qiagen Cell lysis buffer and 10  $\mu$ L Proteinase K were added to each sample. The samples were placed at 55 °C overnight. DNA extraction was performed by phenol-chloroform extraction and ethanol precipitation. DNA purity and amount were determined by using a NanoDrop instrument.

### qPCR

DNA samples were normalized to 100 ng/ $\mu$ L. 500 ng of DNA was used with the TaqMan Gene Expression Master Mix (Life Technologies – Thermo Fisher Scientific, Carlsbad, CA). TaqMan primers were designed and manufactured by Life Technologies – Thermo Fisher Scientific (Carlsbad, CA), Custom Plus TaqMan Assay, Assay ID – AJ6RNJ4. All DNA and reagents were kept on ice. Master mix consisting 10  $\mu$ L of 2 $\times$  reaction mix, 1  $\mu$ L TaqMan assay and 7  $\mu$ L RT-PCR Grade water (Ambion, Life Technologies – Thermo Fisher, Inc., Carlsbad, CA) per sample was prepared. A volume of 5  $\mu$ L (500 ng) DNA was added to Master mix on ice and mixed gently by hand. No template controls were made using RT-PCR Grade water (Ambion). A standard curve was prepared by serially diluting pT2/CaL plasmid into WT C57BL/6 DNA. Samples were run using the impulse setting. The following conditions were used for the qPCR: 95 °C for 10 min and 40 cycles of 90 °C for 15 s then 60 °C for 1 min. See data below in Table S1 where 0.1-1.0 plasmid copies/cell values are background.

**Table 2.** Average number of copies per genome equivalent (cell) of pDNA as measured with qPCR in the liver, brain, spleen, heart, kidney, and lungs of mice treated with polyplexes of pMAT-b-AEMA-2 and pDNA encoded for luciferase via tail vein injection. Plasmid values from 0 – 1.0 are considered background noise.

Tail Vein Injection Details					DNA in Organ (Average Plasmid Copies/Cell)					
	Polymer Dose (mg)	DNA Dose ( $\mu$ g)	N/P	Number of Mice	Liver	Brain	Spleen	Heart	Kidney	Lung
pMAT-b-AEMA-2	200.0	10	7	3	0.01	0.8	1.0	0.7	0.5	0.9
	390.0	10	14	3	0.80	0.9	0.3	0.8	0.1	2.9
	590.0	10	21	3	0.10	0.9	1.3	2.0	0.6	6.5
	490.0	25	7	3	1.60	0.9	0.5	1.0	0.1	1.1
D <sub>5</sub> W	N/A	N/A	N/A	2	0.2	0.1	0.1	0.2	0.1	1.0

## References

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