

SUPPORTING INFORMATION

Novel amphiphilic block-copolymer forming stable micelles and interpolyelectrolyte complexes with DNA for efficient gene delivery

Zeliha Guler Gokce^{1,2}, Semra Zuhul Birol¹, Nataliya Mitina³, Khrystyna Harhay³, Nataliya Finiuk⁴, Valentina Glasunova⁵, Rostyslav Stoika⁴, Sebnem Ercelen^{1*}, Alexander Zaichenko^{3*}

¹ *Center Genetic Engineering and Biotechnology Institute, TUBITAK Marmara Research, Gebze 41470, Kocaeli, Turkey*

² *Department of Nano Science and Nano Engineering Istanbul Technical University, Maslak, 34469, Istanbul, Turkey*

³ *Department of Organic Chemistry, Lviv Polytechnic National University, Lviv 79013, Ukraine*

⁴ *Department of Regulation of Cell Proliferation, Institute of Cell Biology National Academy of Sciences of Ukraine, Lviv 79005, Ukraine*

⁵ *Department of Physical Materials, Donetsk O. O. Galkin Institute of Physics and Engineering National Academy of Sciences of Ukraine, Donetsk 03680, Ukraine*

*Corresponding author: Dr. Alexander Zaichenko; Lviv Polytechnic National University, S. Bandery 12, 79013 Lviv, Ukraine (E-mail address: zaichenk@polynet.lviv.ua, Phone: +38 032 2582390) and Dr. Sebnem Ercelen TUBITAK Marmara Research Center Genetic Engineering and Biotechnology Institute, Gebze 21 41470, Kocaeli, Turkey (E-mail address: sebnem.ercelen@tubitak.gov.tr, Phone: +90 262 6773310).

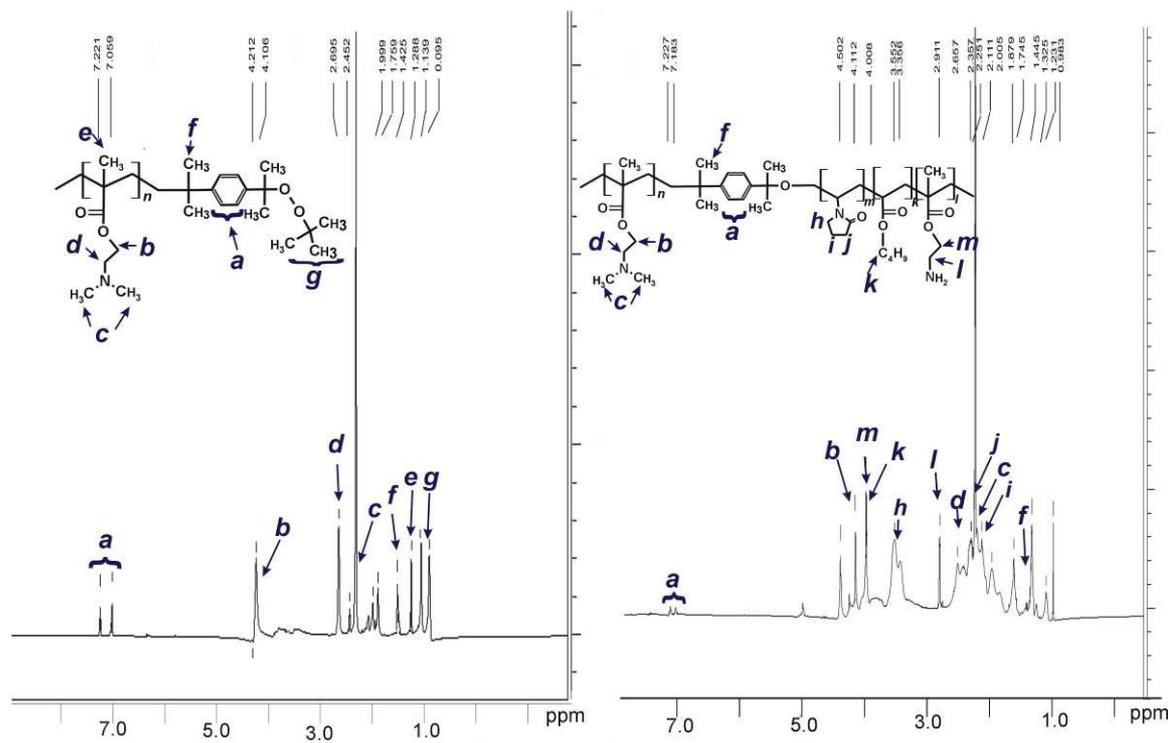


FIGURE S1. ¹H NMR spectra of pDMAEMA-MP (left) and pDMAEMA-*block*-poly(NVP-*co*-BA-*co*-AEM) (right).

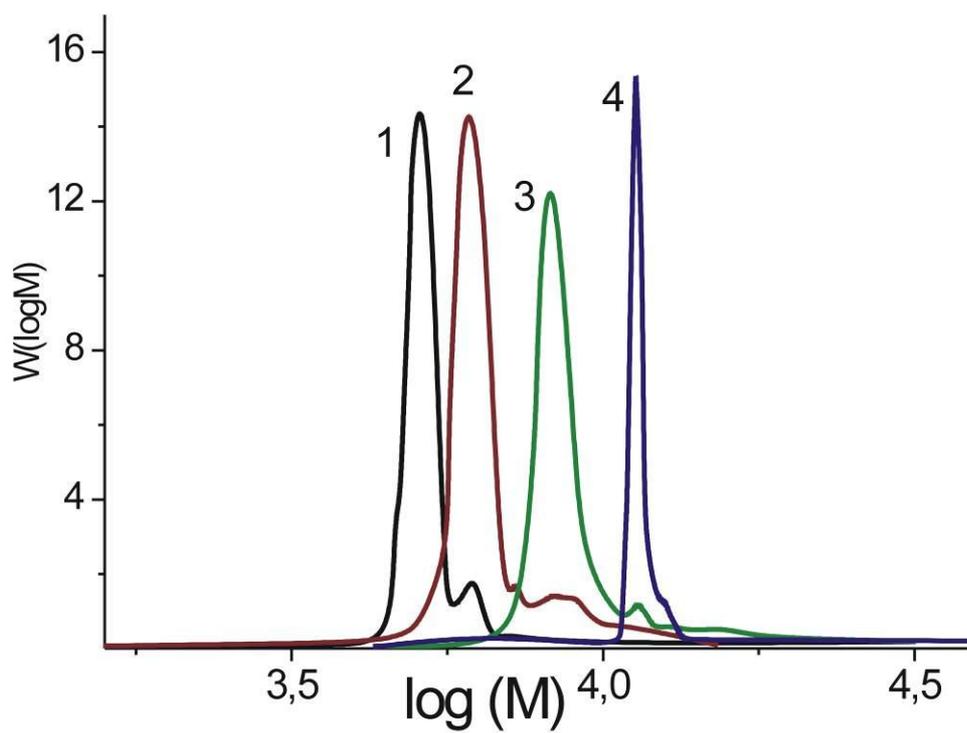


FIGURE S2. Molecular weight distribution of polymers pDMAEMA-MP (1) and pDMAEMA-*block*-poly(NVP-*co*-BA-*co*-AEM)(2-4) : BP81-1(2); BP82-1(3); BP83-1(4);.

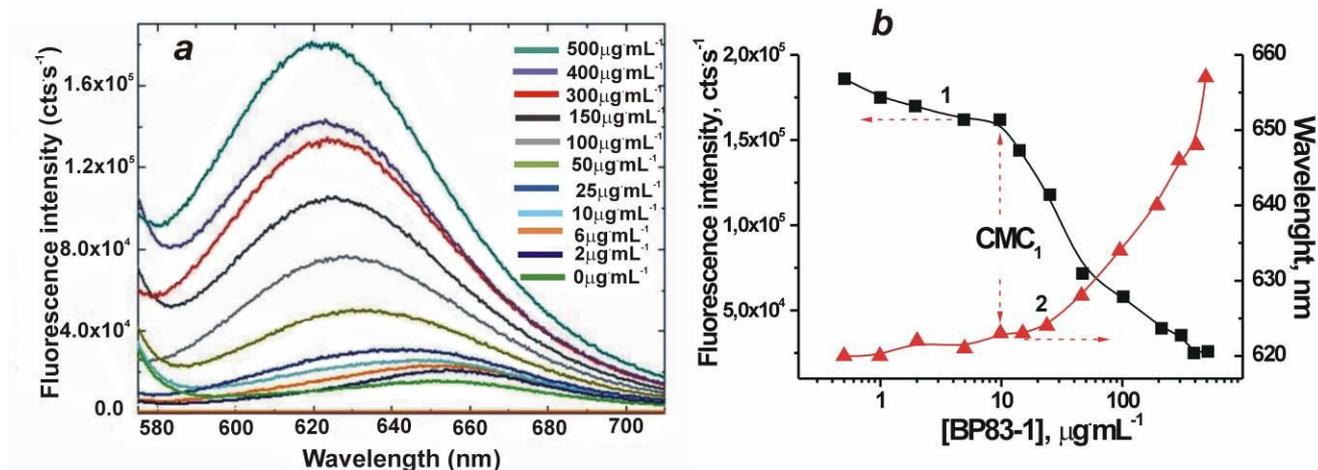


FIGURE S3. Fluorescence emission spectra of Nile Red at different concentrations of the block copolymer BP83-1 (a) and the results of determination of the CMC₁ value of the BP83-1: fluorescence intensity (1) and emission wavelength maximum (2) at increasing concentrations of the BP83-1 (0–500 µg·mL⁻¹) (b).

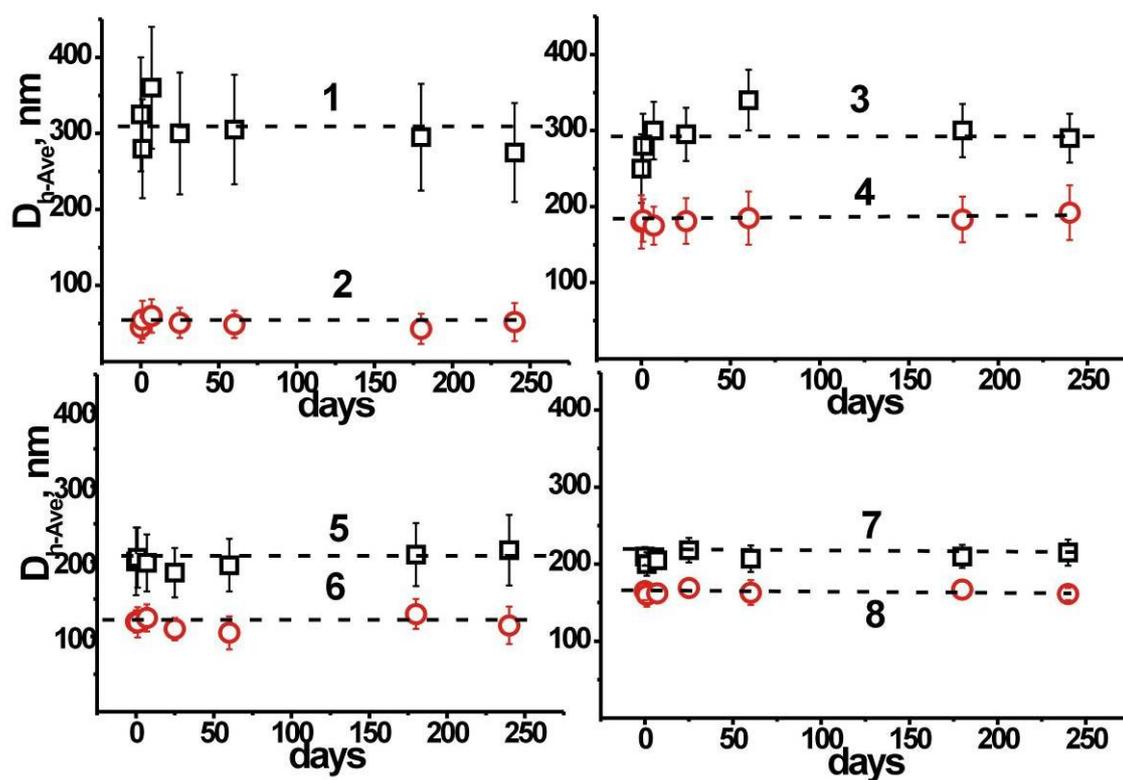


FIGURE S4. Dependence of the hydrodynamic diameter of micelles formed by the poly(DMAEMA)-MP (1,2), BP 83-1 (5,6) and polyplexes poly(DMAEMA)-MP/DNA (3,4) and BP 83-1/DNA (7,8) on time. [pDMAEMA-MP]=1,000 $\mu\text{g}\cdot\text{mL}^{-1}$ (1); 10,000 $\mu\text{g}\cdot\text{mL}^{-1}$ (2) and [BP 83-1]=400 $\mu\text{g}\cdot\text{mL}^{-1}$ (5); 1,000 $\mu\text{g}\cdot\text{mL}^{-1}$ (6); [pDMAEMA-MP/DNA]=100/1,000 $\mu\text{g}\cdot\text{mL}^{-1}$ (3); 1,000/1,000 $\mu\text{g}\cdot\text{mL}^{-1}$ (4) and [BP 83-1/DNA]=100/1,000 $\mu\text{g}\cdot\text{mL}^{-1}$ (7); 1,000/1,000 $\mu\text{g}\cdot\text{mL}^{-1}$ (8).

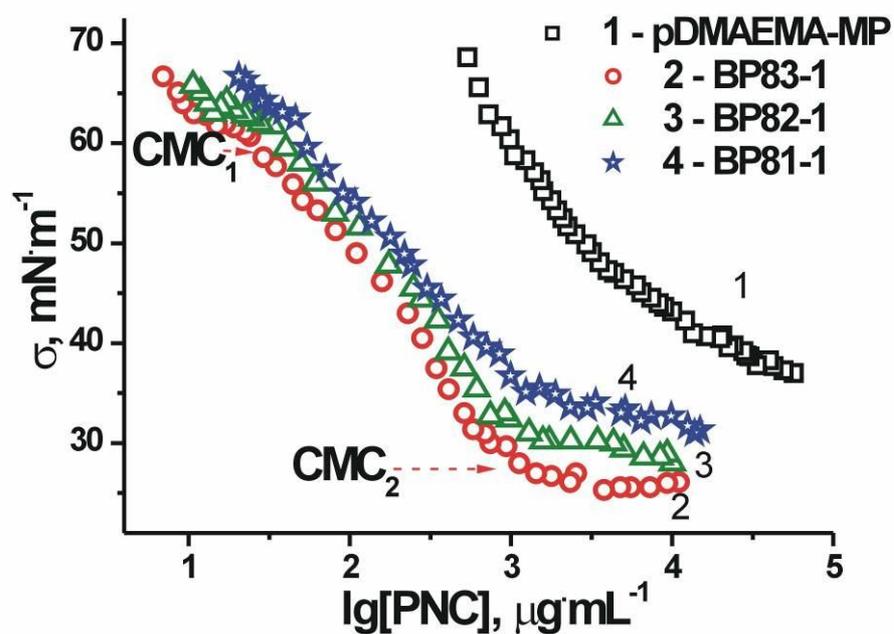


FIGURE S5. Surface tension isotherms of the solutions of pDMAEMA-MP (1) and poly(DMAEMA)-*block*-poly(NVP-*co*-BA-*co*-AEM) with molecular weight of copolymer blocks: 2 – $M_n=11$ kDa (BP83-1); 3 – $M_n=8.2$ kDa (BP82-1); 4 – $M_n=6.7$ kDa (BP81-1).

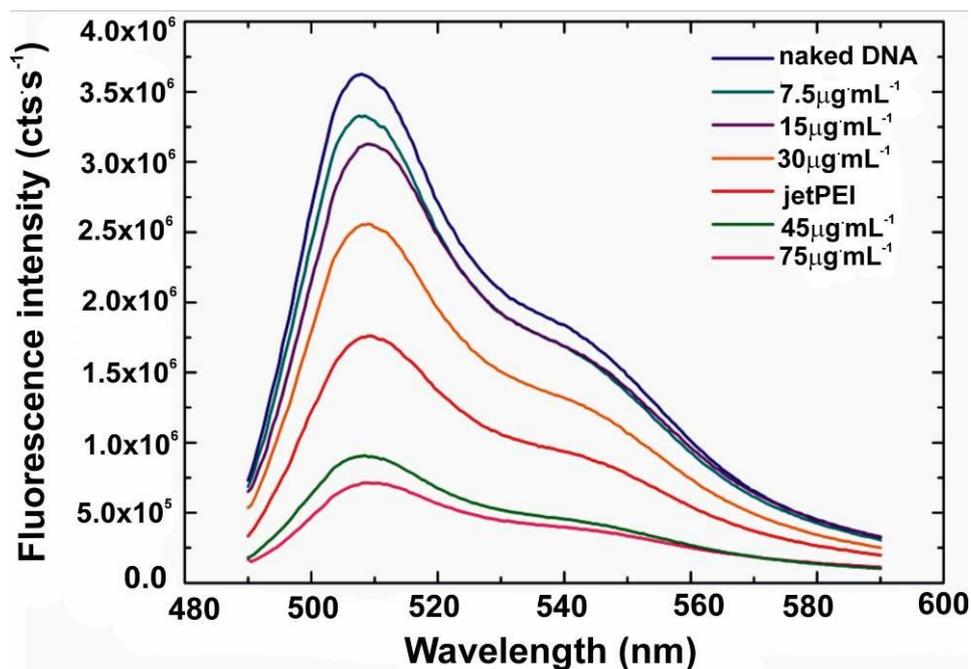


FIGURE S6. Fluorescence emission spectra of YOYO-1 labeled DNA complexed with BP83-1. BP83-1/DNA complexes were formed at BP83-1 concentrations in between $7.5 \mu\text{g}\cdot\text{mL}^{-1}$ – $75 \mu\text{g}\cdot\text{mL}^{-1}$ and 1 mg of DNA in 20 mM MES buffer at pH 7.0. JetPEI/DNA complex was formed with 1 mg of DNA as a control. Free YOYO-1-labeled DNA is marked as 1. The molar ratio of DNA and YOYO-1 was 50:1. The excitation wavelength was 480 nm.

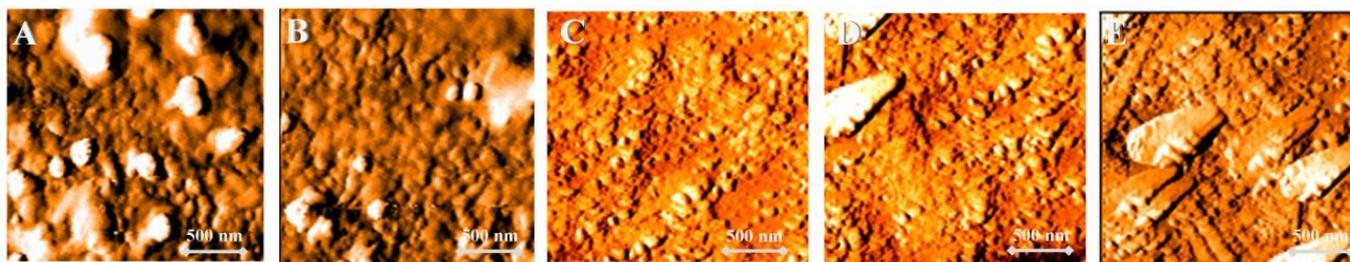


FIGURE S7. AFM images of the BP83-1/DNA complexes that were formed by the BP83-1 at its increasing concentrations $7.5 \mu\text{g}\cdot\text{mL}^{-1}$ (A), $15 \mu\text{g}\cdot\text{mL}^{-1}$ (B), $30 \mu\text{g}\cdot\text{mL}^{-1}$ (C), $45 \mu\text{g}\cdot\text{mL}^{-1}$ (D) and $75 \mu\text{g}\cdot\text{mL}^{-1}$ (E).

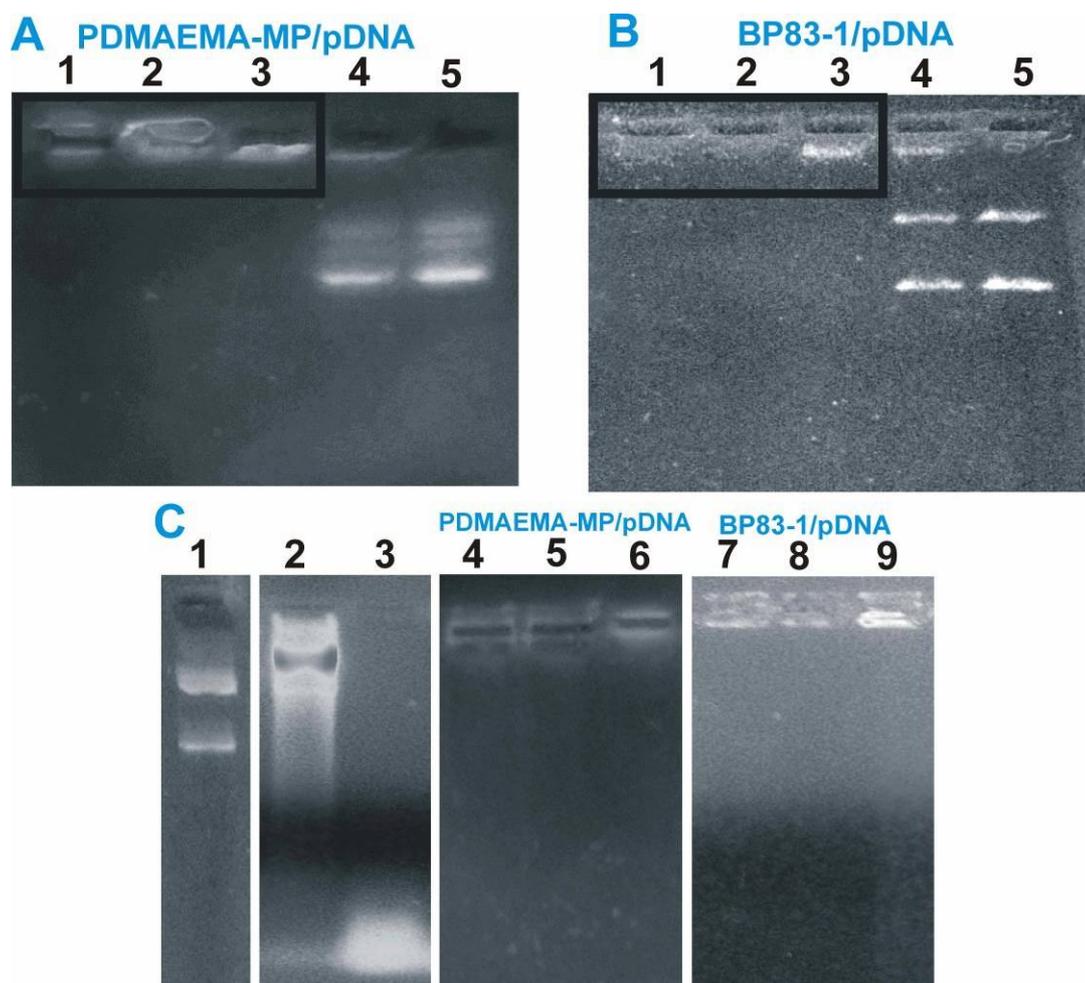


FIGURE S8. Results of gel retardation assay of the PNC (PDMAEMA-MP (A) or BP83-1(B)) and plasmid pEGFP c-1 complexes at electrophoresis in 1% agarose gel. Lines 1 – 10 mg·mL⁻¹ + pDNA; 2 – 1 mg·mL⁻¹ + pDNA; 3 – 100 μg·mL⁻¹ + pDNA; 4 – 10 μg·mL⁻¹ + pDNA; 5 – intact pDNA (black border indicates the complex of PNC/pDNA) (A, B); as well as complex of plasmid DNA with PNC (PDMAEMA-MP (lines 4-6) or BP83-1 (lines 7-9)) (100 μg·mL⁻¹) was treated for 3.5 h with the DNase I in different concentrations: lane 1 – pDNA; 2 – pDNA incubated with DNase I at 0.05 U·μg⁻¹ DNA; 3 – pDNA incubated with DNase I at 0.5 U·μg⁻¹ DNA; 4,7 – PNC/pDNA complex incubated with DNase I at 0.05 U μg⁻¹ DNA; 5,8 – PNC/pDNA complex incubated with the DNase I at 0.5 U·μg⁻¹ DNA; 6,9 – PNC/pDNA complex (C).

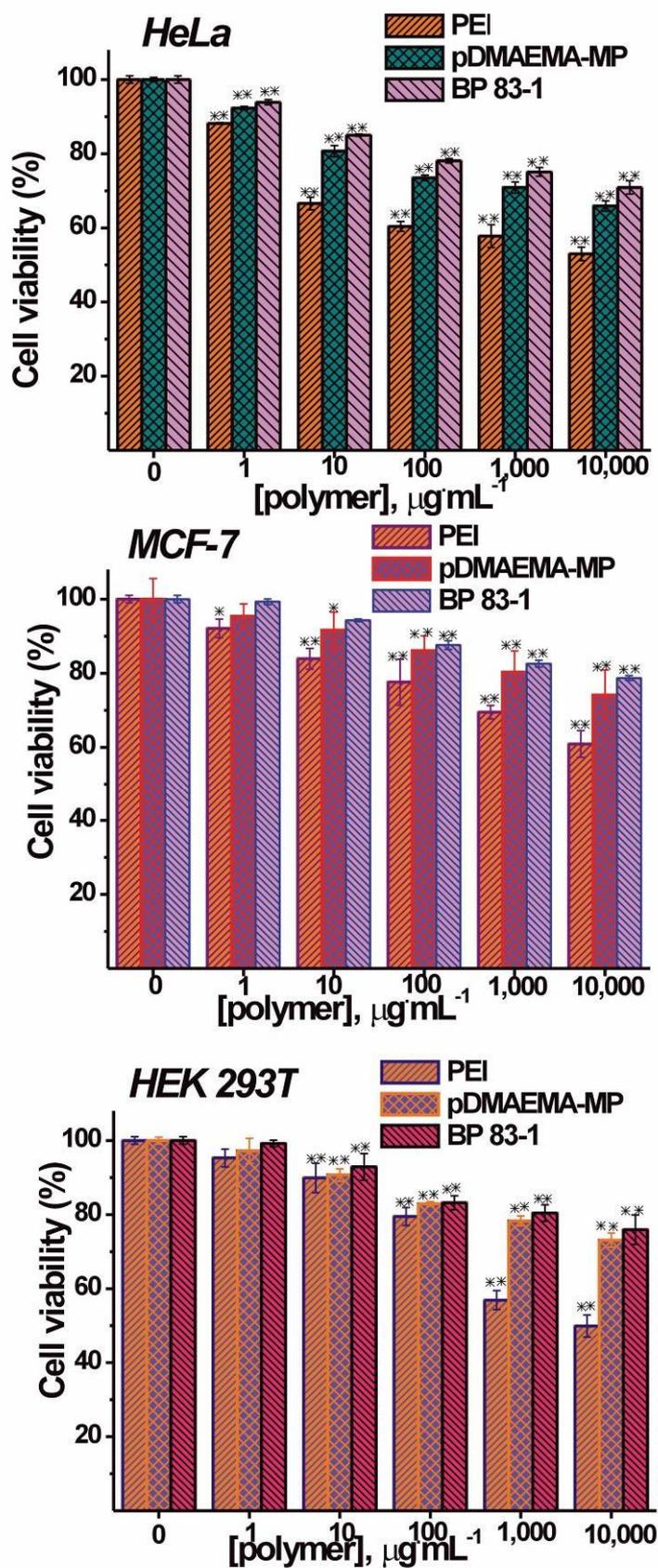


FIGURE S9. Cytotoxicity of PEI and PNC (PDMAEMA-MP and BP83-1) towards *HeLa*, *MCF-7* and *HEK293T* cells. The cytotoxicity of carriers was evaluated by the MTT assay. * – $P \leq 0.05$; ** – $P \leq 0.01$; *** – $P \leq 0.001$ (difference compared with the non-treated cells).

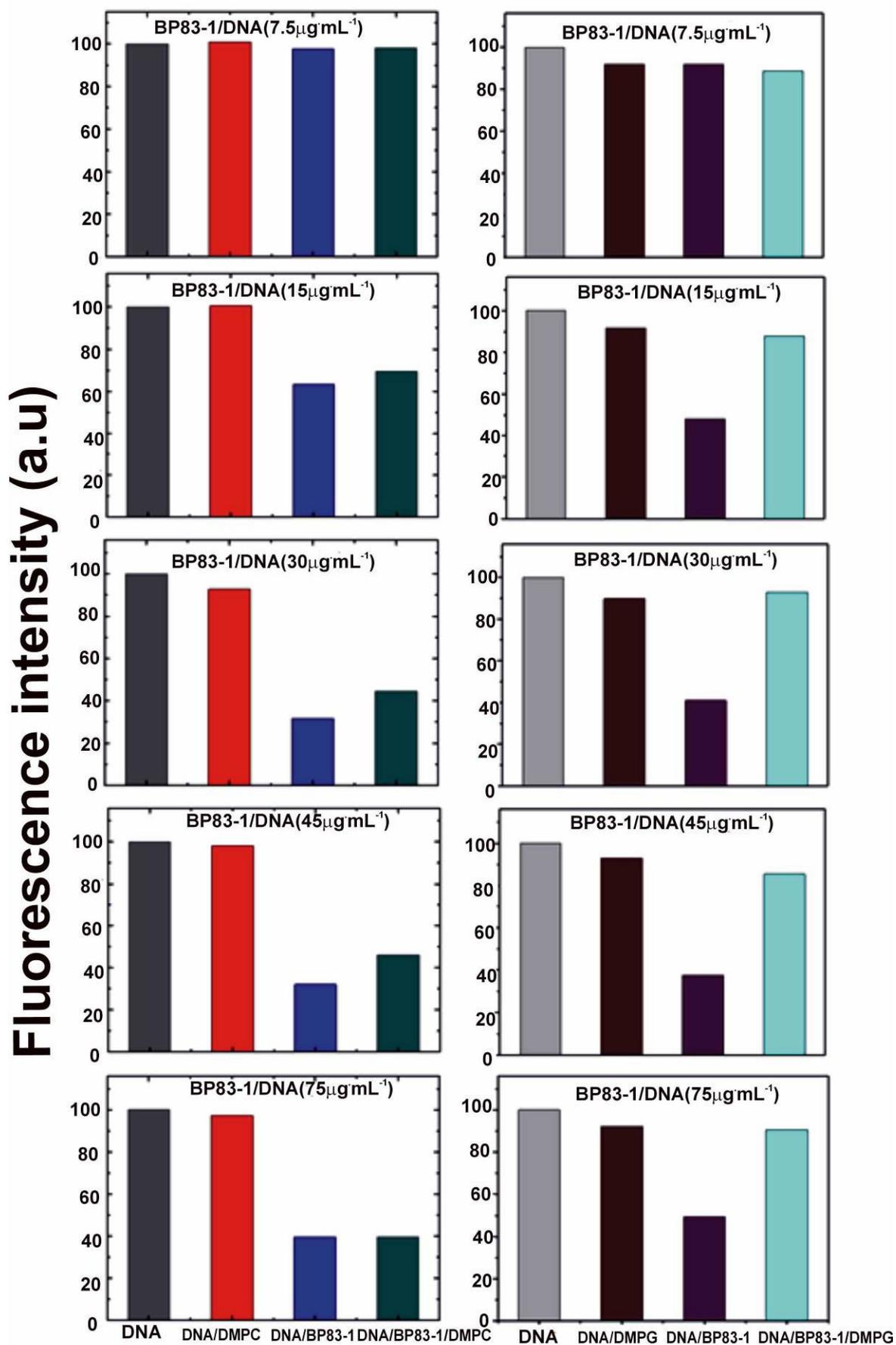


FIGURE S10. Interaction of the BP83-1/DNA complexes with DMPC (*left*) and DMPG (*right*) model lipid vesicles.