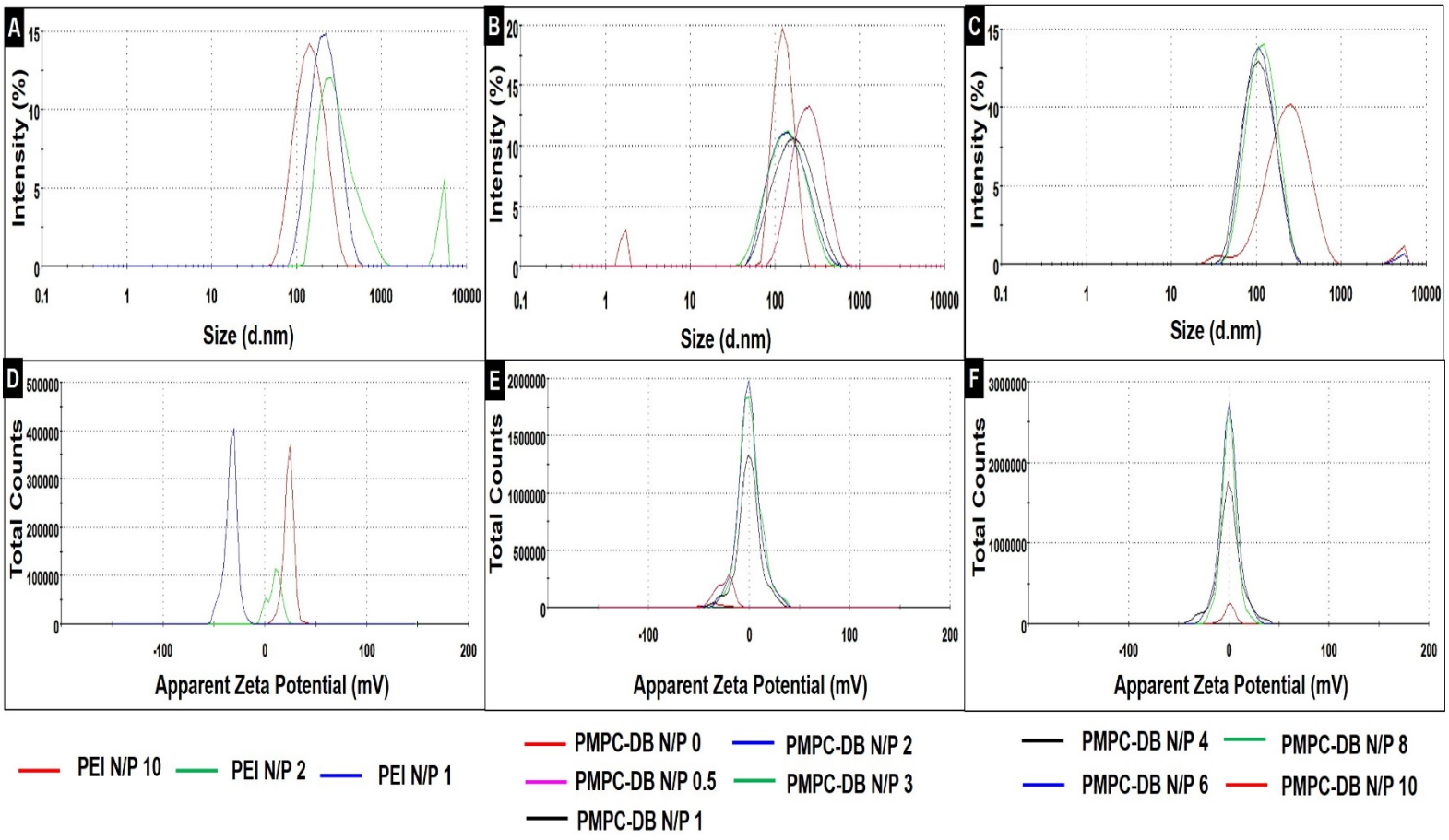
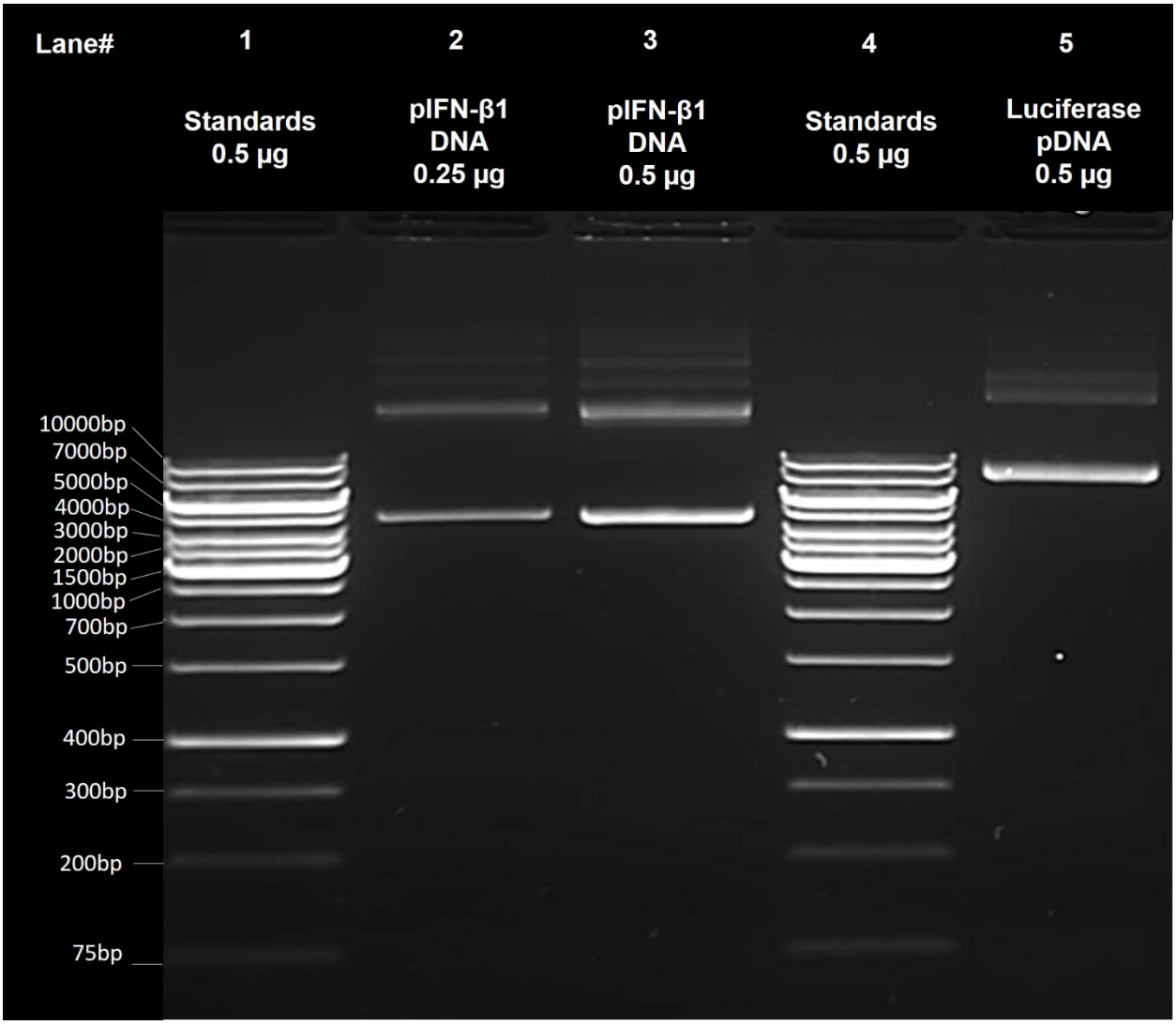
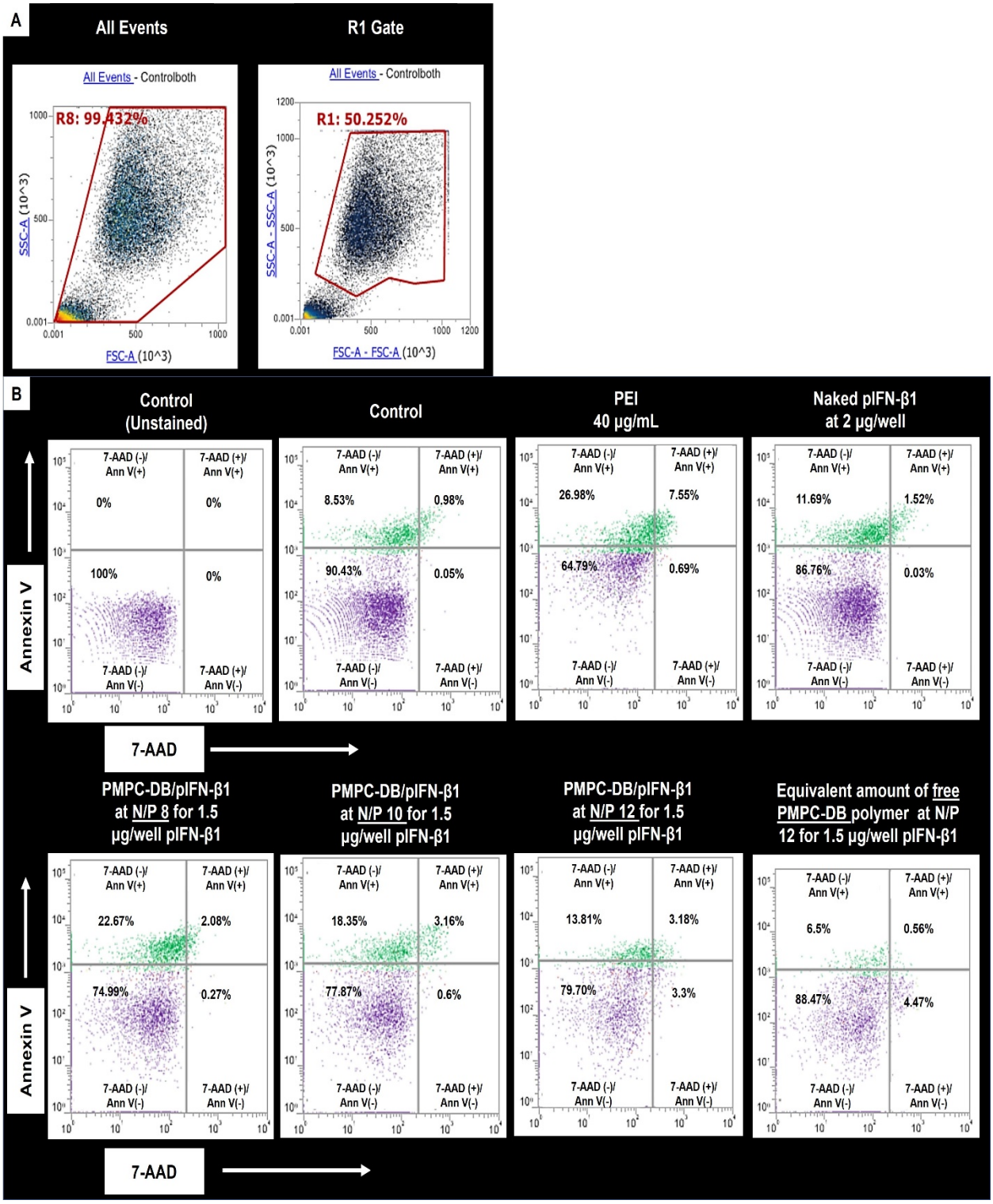


SL Fig. 1: Particle size distribution and apparent zeta potential plots of PMPC-DB/pDNA and PEI/pDNA polyplexes. Particle sizes vs. intensity plots of PEI/pDNA polyplexes in 10 mM HEPES buffer (**A**) and PMPC-DB/pDNA polyplexes in 10 mM phosphate buffer (pH 7.4) at the indicated N/P ratios (**B** and **C**) were measured on a Malvern ZS90 Zetasizer. Total count vs. apparent zeta potential for PEI/pDNA polyplexes (**D**) and PMPC-DB/pDNA polyplexes (**E** and **F**) prepared in 10 mM HEPES buffer, pH 7.4. The plots are representative distributions of the data presented in **Table I** in the main text.

SL Fig. 1 discussion: Particle diameters of the PEI/pDNA and PMPC-DB/pDNA polyplexes at different N/P ratios showed a unimodal distribution in the intensity vs. diameter plots except for PEI/pDNA at N/P 2 and PMPC-DB/pDNA at N/P 0 (free pDNA), 6, and 10. The second minor peak in the polyplex samples is attributed to a very minor proportion of larger particles and we do not believe that this warrants to be classified as a bimodal distribution because according to Rayleigh's scattering principle, the intensity of scattered light is inversely proportional to the fourth power of the wavelength ($I = 1/\lambda 4$). Therefore, even a minor proportion of larger particles will scatter significant amounts of light resulting in a second peak.

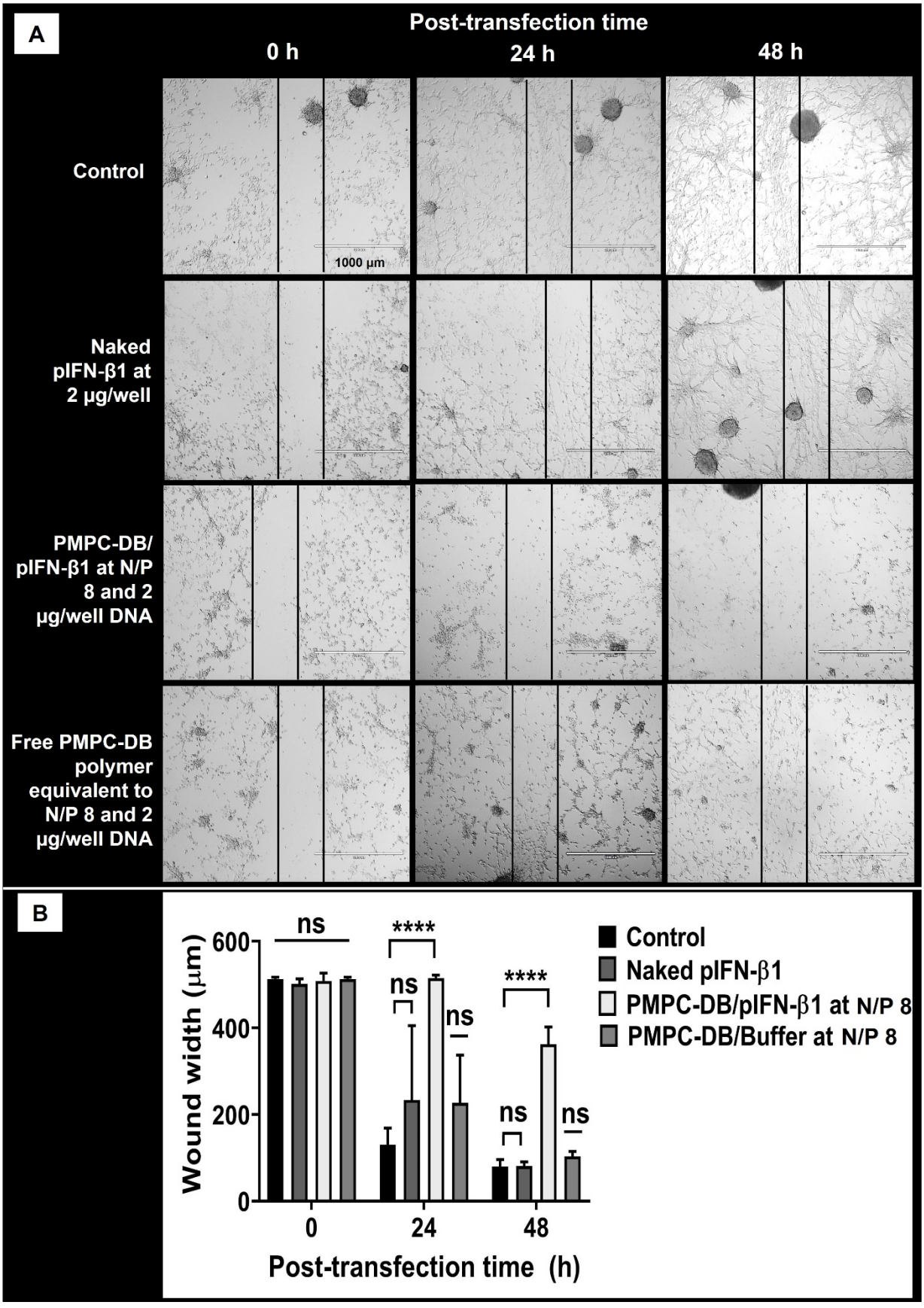


SL Fig. 2: Agarose gel electrophoresis study to evaluate IFN-β pDNA purity. A 0.8% agarose gel containing 0.5µg/mL ethidium bromide was prepared in 0.5X Tris-borate EDTA buffer. DNA ladder was loaded at a 0.5µg/lane amount in lanes 1 and 4. IFN-β pDNA, dissolved in 1X Tris-EDTA buffer pH 7.4, was loaded at 0.25 µg and 0.5µg/lane. The gel was run at 120 V and 100 mA for 50 min. The gel was scanned in an ultraviolet chamber (Proteinsimple red, USA).

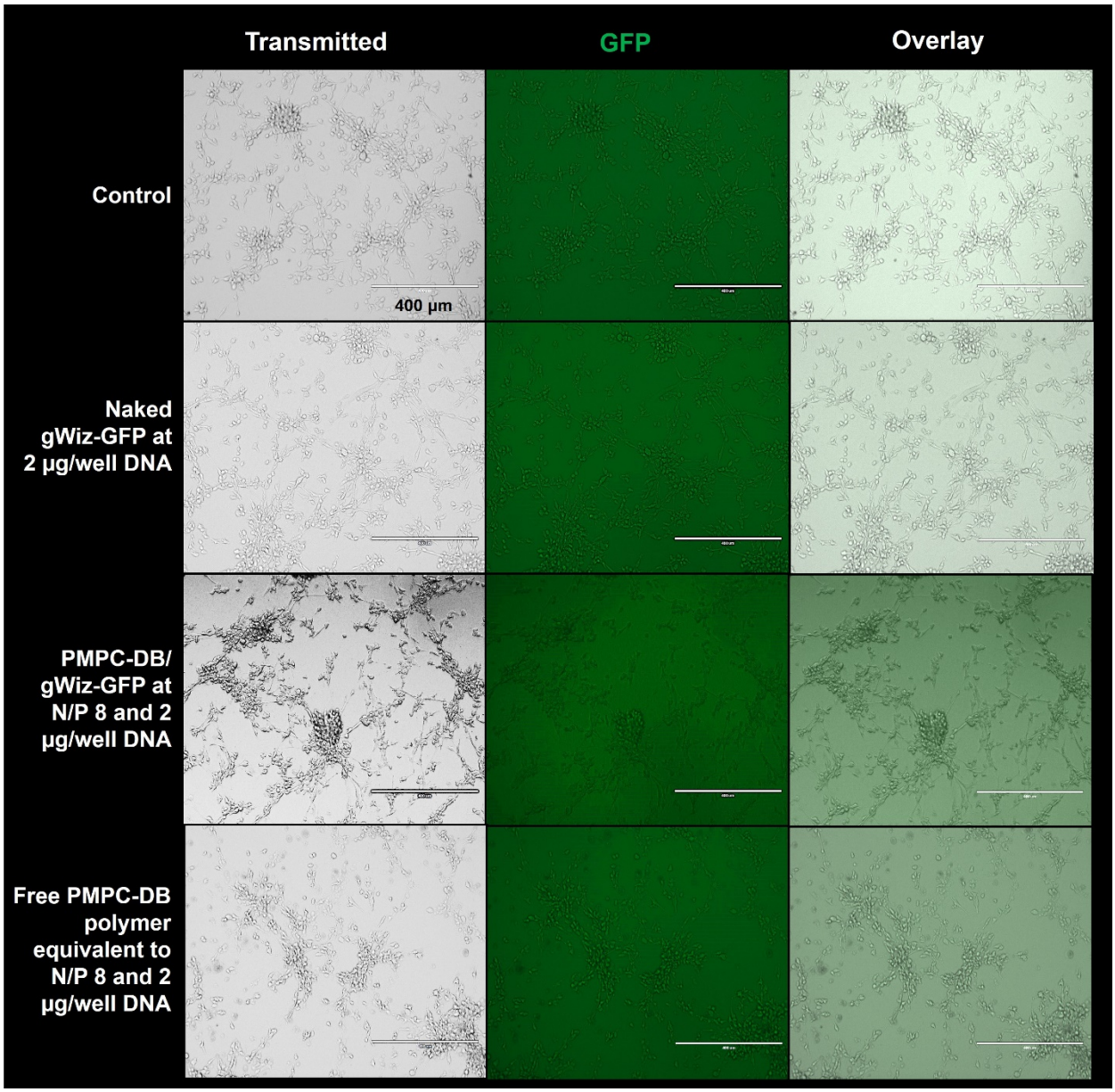


SL Fig. 3: FSC/SSC plots and density plots from flow cytometry analysis for determining the PMPC-DB/IFN-β1 polyplex-induced apoptosis in glioblastoma cells. (**A**) A single-cell suspension of U-87MG cells in annexin binding buffer for each sample was run through an Attune NxT Acoustic Focusing Cytometer, and 50,000 events were recorded in the FSC/SSC dot plot. All events were captured in the polygonal R8 gate, whereas the targeted population (events without dead cells or debris that showed reduced FSC/SSC scattering) were recorded in the R1 gate. The events captured in the R1 gate

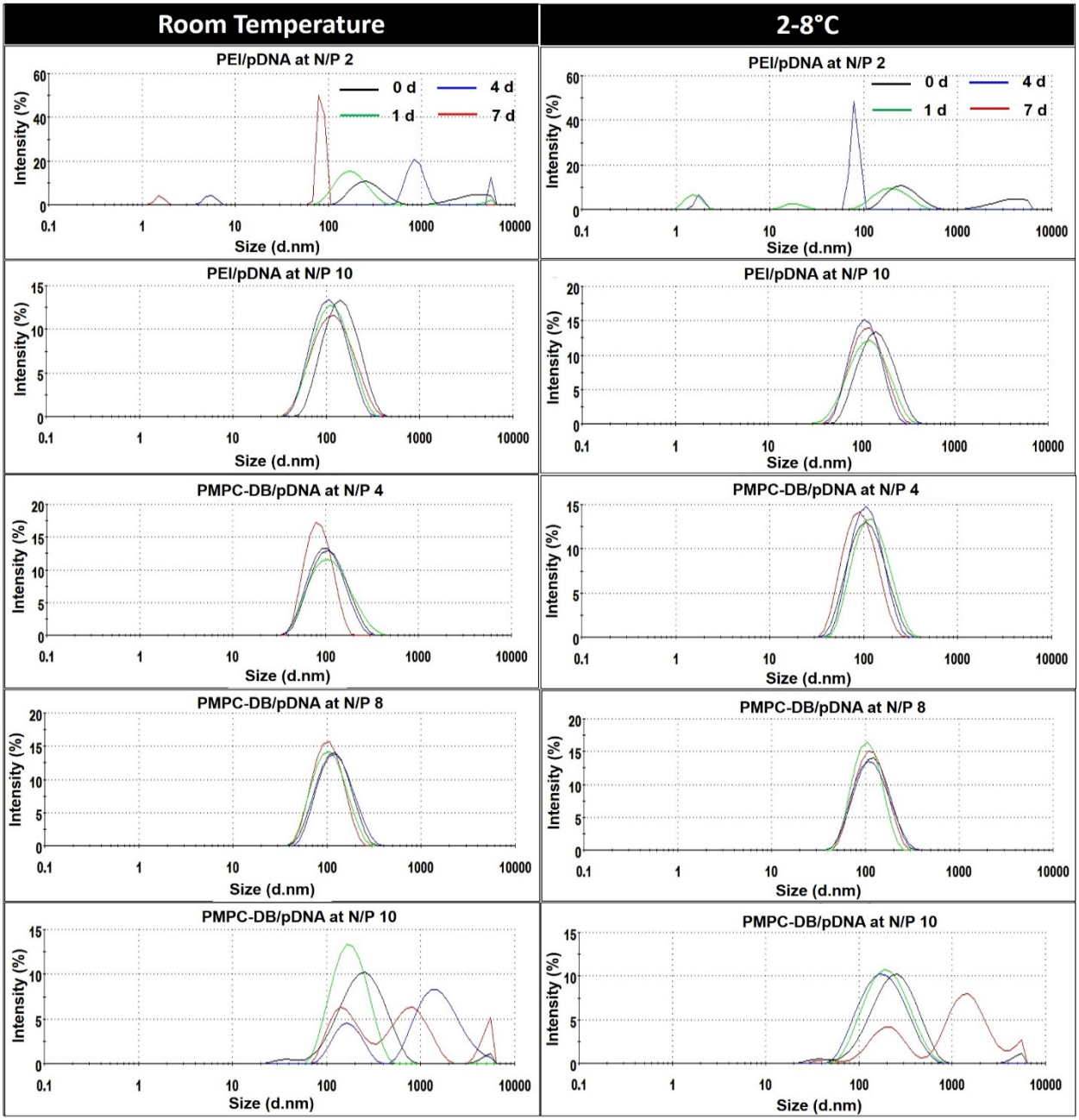
were considered for subsequent analysis. (**B**) Density plots of Annexin V and 7-AAD double-stained U-87MG cells for the indicated samples. Control, unstained sample was used for gating the density plot. Cells were differentiated as viable (7-AAD-/Ann V-), early apoptotic (7-AAD-/Ann V+), or late apoptotic and necrotic cells ((7-AAD+/Ann V+). The percent of viable, early apoptotic or necrotic cells were calculated from the total cellular events captured in the R1 gate.


A
Post-transfection time
0 h
24 h
48 h
Control
1000 µm
Naked pIFN-β1 at 2 µg/well
PMPC-DB/ pIFN-β1 at N/P 8 and 2 µg/well DNA
Free PMPC-DB polymer equivalent to N/P 8 and 2 µg/well DNA
B
Wound width (µm)
600
400
200
0
ns
****
ns
ns
****
ns
ns
0
24
48
Post-transfection time (h)
Control
Naked pIFN-β1
PMPC-DB/pIFN-β1 at N/P 8
PMPC-DB/Buffer at N/P 8

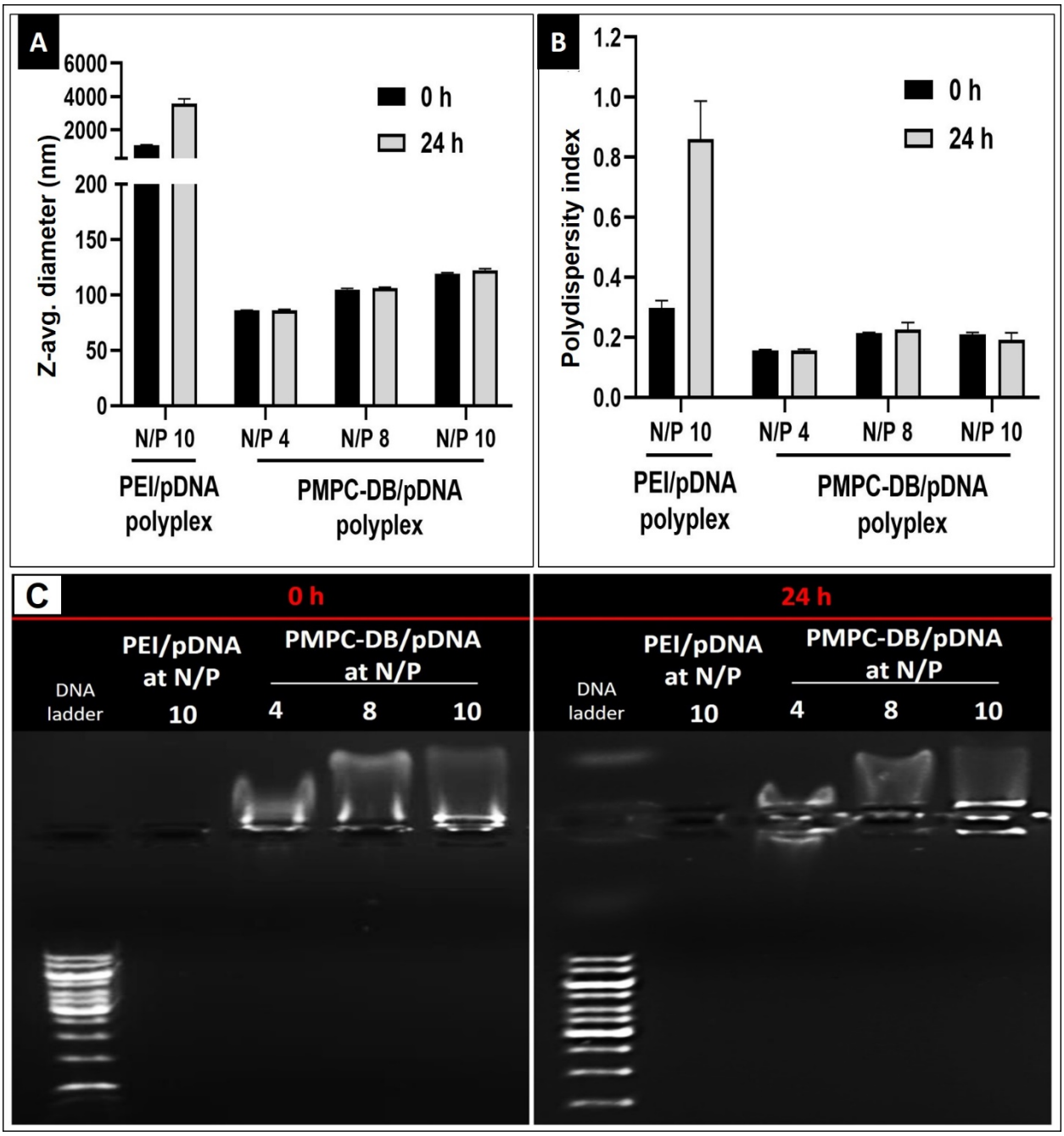
SL Fig. 4: Reproducibility study of the scratch assay demonstrating anti-proliferative and cell migratory inhibition effects of PMPC-DB/IFN-β1 polyplexes in U-87MG cells. (**A**) An artificial scratch was made on confluent U-87MG cells cultured in a 24-well plate. Cells were transfected with naked IFN-β1 DNA, PMPC-DB/IFN-β1 polyplexes at N/P 8 containing 2 μg DNA or free PMPC-DB polymer for 4 h. Control, untreated cells were incubated with fresh MEM/FBS medium. The images of the scratch in each group were acquired at 0, 24, and 48 h post-transfection. As described earlier, the width of the unfilled boundary (wound width) was measured using the Image J software after calibrating the image scale bar. (**B**) The wound width was measured and plotted at 0, 24, and 48 h for control, naked IFN-β1, IFN-β1 polyplexes, and free PMPC-DB polymer. Data are presented as mean ± SD of n=3 measurements at different locations for each sample. Statistical analysis between specified groups was performed using GraphPad Prism 8.4.1. The significance of treated groups was compared against control using one-way ANOVA (Bonferroni's Multiple Comparison Test) and $p<0.0001$ is indicated as ****. ns: non-significant. Scale bar: 1000 μm.



SL Fig. 5: Gene expression efficiency of PMPC-DB/GFP-DNA polyplexes 24 h post-transfection *in vitro*. U-87MG cells were transfected with PMPC-DB/gWiz-GFP DNA at N/P 8 with 2 µg/well DNA for 4 h. Cell treated with naked gWiz-GFP DNA at 2 µg/well, and free PMPC-DB copolymer were used as negative controls. Control, untreated cells were incubated with fresh MEM/FBS medium. Green fluorescence protein expression was observed under a fluorescence microscope 24 h post-transfection. The images were acquired at 20x magnification under transmitted and GFP channel settings. The overlay images were produced using Image J (NIH) software at the same intensity settings for each image. Scale bar: 200 µm.



SL Fig. 6: Particle diameter distribution vs. intensity plots of PMPC-DB and PEI/pDNA polyplexes stored at room temperature and 2-8°C for 7 days. PMPC-DB/pDNA polyplexes containing 20 µg/ml pDNA at N/P 4, 8, and 10 were initially prepared in 10 mM citrate buffer and further diluted 5x using 10 mM phosphate buffer pH 8.0, whereas PEI/pDNA at N/P 2 and 10 polyplexes were prepared in 10 mM HEPES buffer pH 7.4. The samples were stored at room temperature and 2-8°C for the indicated times. Particle diameter was measured either immediately after preparation or 2, 4, and 7 days post-preparation on a Malvern ZS90 Zetasizer. The plots are representative distributions of the data presented in **Fig. 10** in the main text.



SL Fig. 7: Stability of PMPC-DB/pDNA polyplexes in 0.15 M NaCl. PMPC-DB/pDNA polyplexes containing 20 µg/ml pDNA at N/P 4, 8, and 10 were initially prepared in 10 mM citrate buffer and further diluted 5x using 10 mM phosphate buffer pH 8.0, whereas PEI/pDNA at N/P 10 polyplexes were prepared in 10 mM HEPES buffer pH 7.4. The samples additionally contained 0.15 M NaCl and were stored at room temperature for 24 h. (**A**) Z-average particle diameters and (**B**) polydispersity indices of DNA polyplexes were measured immediately after preparation (0 h) and at 24 h post-preparation using dynamic light scattering. Data are presented as mean±SD of n=3 measurements. (**C**) DNA retention in PEI/pDNA and PMPC-DB/pDNA polyplexes containing 0.15 M NaCl at different N/P ratios were studied using agarose gel electrophoresis. Polyplexes containing 0.4 µg pDNA were loaded into a 0.8%

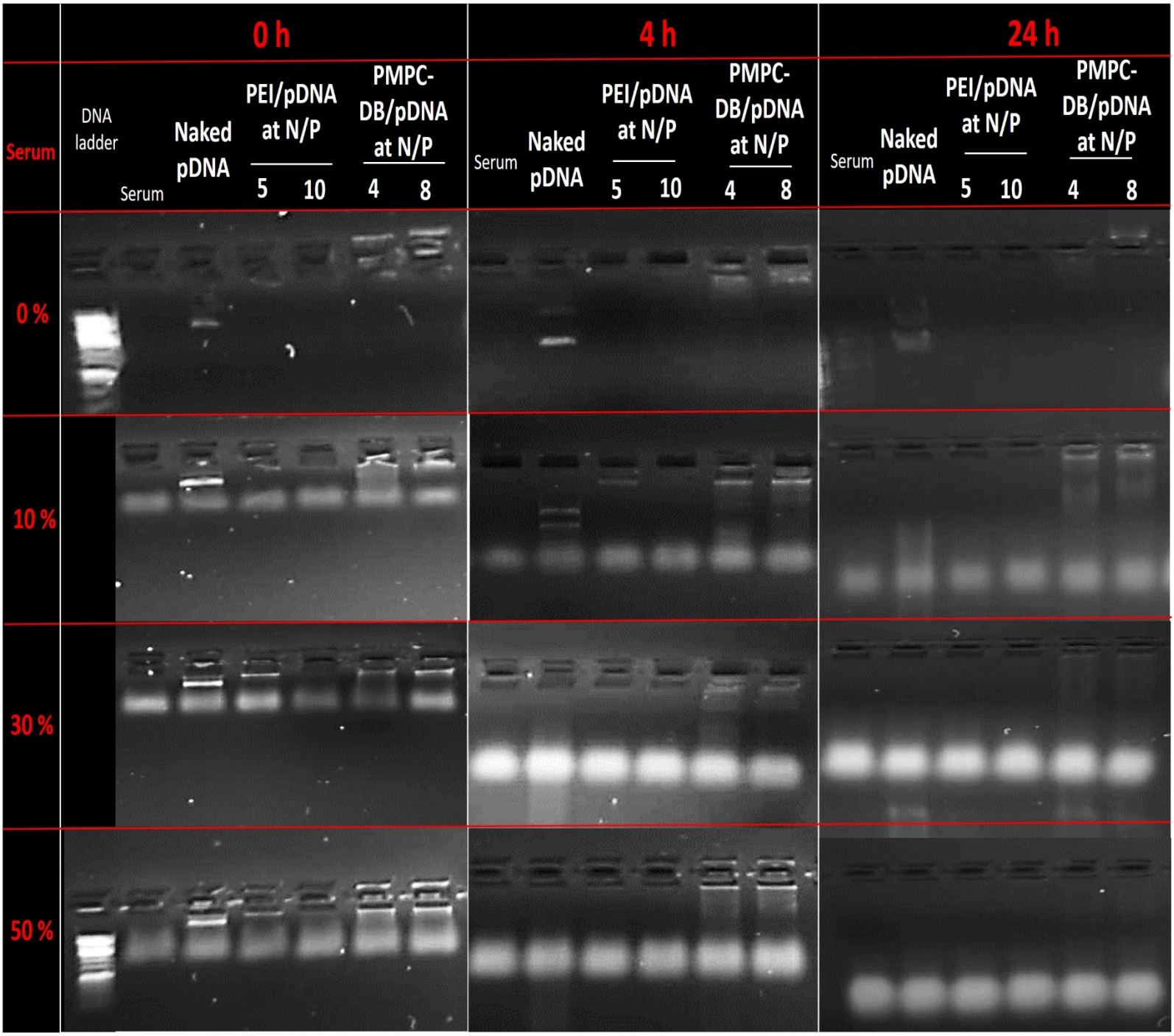
w/v agarose gel containing 0.5 µg/mL ethidium bromide. The gel was run at 120 V/100 mA for 75 min and was scanned under UV using a ProteinSimple red imaging system.

SL Fig. 7 discussion. Stability of PMPC-DB/pDNA complexes in high salt conditions

The presence of proteins and salts in physiological conditions facilitate the immediate aggregation of pDNA complexes after intravenous administration and minimizes their stability (6, 21). Wan *et al*. have studied the effect of salt on the polyplex stability suggesting that higher salt concentrations disassembled polyplexes formed using high molecular weight polypeptides containing disulfide bonds in the backbone, and the DNA molecules were released at a faster rate (22, 23). Incubation of polyplexes under high salt concentrations led to immediate disassociation of DNA from the polymer, loss of excess oligocations, and rearrangement of oligocations within the particles (22). Therefore, we evaluated the stability of PMPC-DB polyplexes at N/P 4, 8, and 10 in a buffer containing 0.15 M NaCl. PEI/pDNA at N/P 10 polyplexes were used for comparison. Immediately and 24 h post-dilution, the average particle diameter and PdI of the polyplexes were measured using dynamic light scattering, whereas the ability of the polyplexes to retain DNA was evaluated using agarose gel electrophoresis.

The Z-average particle diameter and PdI of polyplexes were shown in **SL Fig. 7A-B**. Immediately after incubation, PMPC-DB/pDNA at N/P 4, 8, and 10 showed an average particle diameter of 85.9±0.1, 104.8±1.3, 119.4±0.8 nm respectively with a narrow PdI of <0.22 (**SL Fig. 7A-B**). There were no considerable differences in particle diameter and PdI at 24 h post-incubation. Interestingly, PEI/pDNA at N/P 10 showed an average particle diameter of 1073.7±40.5 nm which further increased to 3600±264.5 nm 24 h post-salt incubation. Our results align with the previously reported results using PMPC-DB/siRNA polyplexes where the 20k PMPC corona-containing siRNA polyplexes appeared most resistant to higher salt concentration-mediated destabilization (3). Our data suggest that PMPC coronas improve the stability in higher ionic strength conditions. The likely reason for the stability of PMPC-DB polyplexes in high salt conditions is due to the steric stabilization effects of the zwitterionic PMPC corona whereas PEI polyplexes are stabilized by electrostatic forces alone, and therefore they aggregate more rapidly under high salt conditions (6).

Agarose gel electrophoresis studies confirmed that PMPC-DB/pDNA polyplexes at N/P 4, 8, and 10 showed complete DNA condensation immediately and 24 h post-incubation in a buffer containing 0.15 M NaCl (**SL Fig. 7C**). Despite a larger particle diameter, PEI/pDNA at N/P 10 containing 0.15 M NaCl did not show any free DNA in gel electrophoresis and the absence of free DNA bands in the loading well suggested that PEI/pDNA at N/P 10 completely condensed DNA. In conclusion, PMPC-DB/pDNA polyplexes at N/P 4, 8, and 10 showed resistance to polyplex disassembly and particle aggregation in high salt conditions.



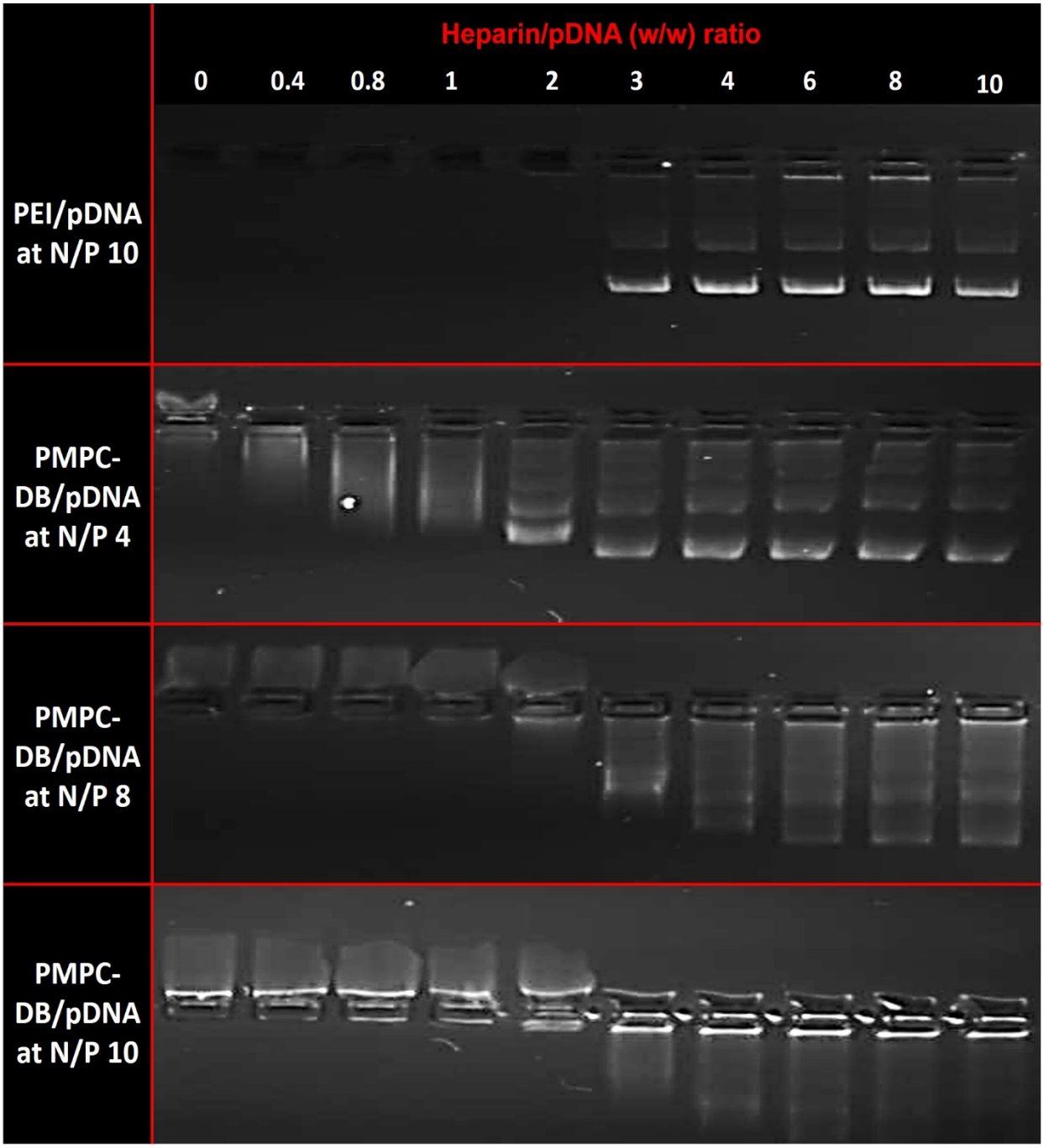
SL Fig. 8: Stability of PMPC-DB/pDNA and PEI/pDNA polyplexes in serum-containing medium studied using agarose gel electrophoresis. PMPC-DB/pDNA polyplexes containing 20 µg/ml pDNA at N/P 4, 8, and 10 were initially prepared in 10 mM citrate buffer and further diluted 5x using 10 mM phosphate buffer pH 8.0, whereas PEI/pDNA at N/P 10 polyplexes were prepared in 10 mM HEPES buffer pH 7.4. The samples contained 0, 10, 30, and 50 % fetal bovine serum in phenol red-free DMEM cell culture medium. The samples were incubated at 37°C for 4 and 24 h. Polyplexes containing 0.4 µg pDNA were loaded into a 0.8% w/v agarose gel containing 0.5 µg/mL ethidium bromide. Each gel was

also loaded with serum in DMEM medium at the indicated concentrations and naked pDNA at 0.4 μg/lane. The gel was run at 120 V/100 mA for 75 min and was scanned under UV using a ProteinSimple red imaging system.

SL Fig. 8 discussion. Stability of PMPC-DB/pDNA polyplexes in serum-containing media

The stability of PMPC-DB/pDNA polyplexes in the serum-containing cell culture medium was evaluated using agarose gel electrophoresis. PMPC-DB/pDNA polyplexes at N/P 4 and 8 were incubated with 0, 10, 30, and 50% serum-containing phenol red-free DMEM medium at 37°C for 4 and 24 h. Naked pDNA and PEI/pDNA at N/P 5 and 10 were used for comparison. Cell culture medium supplemented with serum at the indicated concentrations were also subjected to agarose gel electrophoresis. The gel images at different serum concentrations and incubation times were demonstrated in **SL Fig. 8.**

In serum-free cell culture medium, PMPC-DB/pDNA at N/P 4 and 8 showed complete DNA condensation up to 4 h incubation. The relative intensity of DNA bands near the loading wells were reduced at 24 h. PEI/pDNA at N/P 5 and 10 showed complete DNA condensation up to 24 h incubation time in serum-free medium. Naked pDNA remained stable for 4 h, however, the intensity at 24 h was considerably reduced suggesting a potential degradation in the serum-free culture medium. **In 10% serum-containing cell culture medium**, PMPC-DB/pDNA polyplexes showed a considerable DNA condensation up to 4 h which was slightly reduced at 24 h incubation. Naked pDNA remained stable for 4 h, but likely degraded at 24 h incubation time in 10% FBS/DMEM medium indicated by the presence of a smear-like pattern near the serum-specific bands. **In 30 and 50% serum-containing medium,** PMPC-DB/pDNA polyplexes showed DNA condensation up to 4 h demonstrated by the presence of DNA staining near the loading wells but showed DNA degradation at the 24 h incubation time. Therefore, it can be concluded that PMPC-DB/pDNA polyplexes showed considerable DNA condensation for 4 h when incubated in serum-free cell culture medium, and 50% serum-containing medium—a condition that somewhat simulates an *in vivo* environment (24). Our data is in agreement with the previously reported serum stability observations by our coworkers, Jackson *et al*. who demonstrated that PMPC-DB/siRNA polyplexes were stable in 10% and 30% FBS for 100 min (3). Naked pDNA rapidly degraded in 50% FBS/DMEM in less than 4 h, also in agreement with previous reports (24, 25). **Noteworthy,** t**he stability of polyplexes in 10% serum-containing medium correlated well with the observed *in vitro* transfection efficiency of the PMPC-DB/pDNA polyplexes**. The observed stability of PMPC-DB/pDNA polyplexes at N/P 4 and 8 in 10% serum-containing medium is a likely factor contributing to their *in vitro* activity. The highest luciferase expression were mediated by N/P 4 and N/P 6 polyplexes in U-87 MG and U-138 MG cells, respectively, and the highest anti-proliferative and apoptotic effects were mediated using PMPC-DB/pIFN-β1 DNA polyplexes at N/P 8. These results support the notion that the physicochemical characteristics of DNA polyplexes are a useful predictor of their biological performance *in vitro*.



SL Fig. 9: Stability of PMPC-DB/pDNA polyplexes against heparin competition studied using agarose gel electrophoresis. PMPC-DB/pDNA polyplexes containing 20 µg/ml pDNA at N/P 4, 8, and 10 were initially prepared in 10 mM citrate buffer and further diluted 5x using 10 mM phosphate buffer pH 8.0, whereas PEI/pDNA at N/P 10 polyplexes were prepared in 10 mM HEPES buffer pH 7.4. The samples were incubated with heparin at heparin/pDNA (w/w) ratios ranging from 0 to 10 at 37°C for 30 min. Post-incubation, the samples were immediately electrophoresed on a 0.8% w/v agarose gel containing 0.5 µg/mL ethidium bromide. The gel was run for 60 min at 120 V/100 mA and was scanned under UV using a ProteinSimple red imaging system.

SL. Fig 9 discussion. Stability of PMPC-DB/pDNA polyplexes demonstrated using a heparin competition assay

Heparin is a linear polysaccharide containing repeating units of highly sulfated uronic acid D-glucosamine disaccharide that confers it a high negative charge (26). Apart from serum proteins in the blood, anionic heparan sulfates present in the glomerular basement membrane of the kidney play a major role in decomplexation-mediated instability of DNA polyplexes *in vivo* due to charge-based ionic interactions between the positively charged components of DNA polyplexes and negatively charged heparan sulfates (3).

To determine the impact of particle surface chemistry on polyplex stability, PMPC-DB/pDNA (20 μg/mL) at N/P 4, 8, and 10 were incubated with heparin solution at heparin/pDNA (w/w) ratios ranging from 0 to 10 at 37°C for 30 min. PEI/pDNA at N/P 10 was used for comparison. Post-incubation, the samples were immediately electrophoresed on a 0.8% w/v agarose gel and was imaged on a UV illuminator. The gel images for the stability of PMPC-DB/pDNA polyplexes by heparin competition assay were shown in **SL Fig. 9**. PMPC-DB/pDNA at N/P 4 partially dissociated in the presence of heparin up to heparin/pDNA ratio 1, whereas the polyplexes completely destabilized at ratios 2 or higher (**SL Fig. 9**). PMPC-DB/pDNA at N/P 8 was stable at the heparin/pDNA ratio up to 2, followed by partial dissociation at ratios 3 or higher. Interestingly, PMPC-DB/pDNA at N/P 10 showed complete DNA condensation across the entire range of heparin/pDNA ratios of 0-10 suggesting greater resistance to charge-induced destabilization. PEI/pDNA at N/P 10 was stable up to a heparin/pDNA ratio of 2, but completely destabilized at ratios >3. At the same N/P ratios, PMPC-DB polyplexes showed greater resistance to charge-induced destabilization compared to PEI polyplexes.