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Supporting Information

# A Catch-and-Release Nano-based Gene Delivery System

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# 1. Experimental Section

## 1.1. Chemicals, Reagents and Kits

Agarose (Sigma-Aldrich), CellMask™ Deep Red Dye (ThermoFisher/Invitrogen), dimethyl sulfoxide (DMSO, Sigma-Aldrich 99), dopamine hydrochloride (Sigma-Aldrich, 98 %), Dulbecco's Modified Eagle Medium (DMEM, Gibco), Dulbecco's phosphate buffered saline (DPBS, Gibco), Fetal bovine serum (FBS, Gibco), Hoechst 33342 (Sigma-Aldrich, 98%), Orange-G loading dye (10X, glycerol-based, Alfa Aesar), penicillin-streptomycin (pen-strep, Sigma Aldrich), poly-L-arginine (Mw = 5000 – 15000 Da, Sigma-Aldrich), poly-L-histidne (Mw = 5000 – 25000 Da, Sigma-Aldrich), ProLong™ Diamond Antifade Mountant (ThermoFisher Scientific), PureLink™ Quick Plasmid Miniprep Kit (K210010, Invitrogen), TrypLE™ Express Enzyme (1X, no phenol red, Gibco).

## 1.2. Synthesis of PDA NPs

Tris(hydroxymethyl)aminomethan (135 mg, 1.11 mmol) was dissolved in 195 mL Milli-Q water and stirred for 30 min at room temperature. Dopamine hydrochloride (90 mg, 0.47 mmol) was dissolved in 1.00 mL Milli-Q water and added dropwise to the Tris solution. The mixture was stirred overnight and purified by centrifugation at 4000 rpm for 10 min on a Thermo Scientific Sorvall LYNX 4000 Superspeed Centrifuge to remove larger aggregates from the desired PDA NPs. The supernatant suspension was centrifuged at 15000 rpm for 20 min. The resulting pellet was washed with Milli-Q water to remove excess reagents (3x). The particles were frozen in liquid nitrogen and lyophilised, using a Telstar LyoQuest benchtop freeze dryer (0.008 mBar, −70 °C), to obtain the NP concentration.

## 1.3. Synthesis of pArg-PDA and pHis-pArg-PDA NPs

The PDA NP suspension (1 mL, 1 mg/mL) was added to a stirred mixture of 100 mM aqueous $NH_4OH$ (250 µL) in 10 mL Milli-Q water. Various amounts of pArg (dissolved in Milli-Q water 1 mL) and pHis (resuspended in DMSO 1 mL) were added dropwise to the PDA NP suspension, according to Table S1. The reaction was stirred overnight at room temperature. For purification, the mixture was centrifuged at 12000 rpm for 15 min using a Sigma 1-14 Microfuge and washed with Milli-Q water (3x). The concentration of all nanocarrier formulations has been determined based on PDA NP concentration.

**Table S1.** Amount of pArg and pHis used for the preparation of pArg-PDA and pHis-pArg-PDA NPs.

| Compound | m(pArg)/mg | m(pHis)/mg |
|---|---|---|
| **pArg-PDA** | 10.0 | 0.0 |
| **pHis-pArg-PDA_1** | 5.0 | 5.0 |
| **pHis-pArg-PDA_2** | 3.2 | 6.8 |
| **pHis-pArg-PDA_5** | 1.6 | 8.4 |

## 1.4. Dynamic light scattering

DLS and zeta potential measurements were recorded using a Zetasizer Nano ZS instrument (Malvern Panalytical, UK) with a sample concentration of 0.01 mg/mL. Milli-Q water was used as the dispersion medium. All Measurements were conducted three times with 15 subruns for each sample. Error bars represent the standard deviation of three measurements. Zeta potential was measured in a folded capillary Zeta cell (DTS1070, Malvern, UK).

## 1.5. Scanning electron microscopy (SEM)

SEM images were obtained using a TESCAN MIRA3 field emission gun scanning electron microscope (FEG-SEM). The instrument was operated at 1.00 – 5.00 kV accelerating voltage using the in-beam SE detector. Samples were prepared as aqueous suspensions and drop-casted (4 drop sites, 3 µL each) on square 21 mm microscopy glass slides. After 24 h of drying under ambient conditions, the slides were Pt-coated on a Quorum Technologies Q150T ES Turbo-Pumped Sputter Coater prior to imaging. ImageJ was used to determine the diameter of 175 NPs and the size is given as mean ± standard deviation.

### 1.6. Plasmid-EGFP-C1 DNA (EGFP pDNA) Amplification in Bacterial Culture

The plasmid amplification was conducted in *E. coli* DH5α, provided by Prof Heike Laman's laboratory at the Department of Pathology, University of Cambridge. Miller's LB broth containing 50 µg/mL kanamycin was inoculated with a single colony and grown at 37 ºC for 16 h with shaking at 225 rpm. pEGFP-C1 was isolated using the PureLinkTM Quick Plasmid Miniprep Kit (Invitrogen), following the manufacturer's protocol. For size analysis, 300 ng of pEGFP-C1 was linearized using 10 units of HindIII-HF restriction enzyme (New England Biolabs, R3104M) at 37 ºC for 1 h. The linearized plasmid was visualized on a 0.8% agarose gel, alongside a 1 Kb plus DNA ladder (Invitrogen, 10787018) and 300 ng of undigested DNA.

### 1.7. Gel Electrophoresis of pDNA@pArg-PDA and pDNA@pHis-pArg-PDA NPs

To evaluate the pDNA immobilisation to the NPs gel electrophoresis with 1% agarose gels in 1X Tris-acetate-EDTA (TAE) buffer in a Bio-Rad Sub-Cell electrophoresis system were conducted. SYBR Safe stain (4 µL) were added to the hot agarose solution (60 mL) prior to casting. For immobilisation studies, various volumes (0.3, 0.9, 1.8, 3.0, 4.5, 9.0 and 15 µL) of 1.00 mg/mL NP stock solutions were prepared and diluted with Milli-Q water to a total volume of 15 µL. The samples were incubated with 300 ng of pDNA for 15 min. Prior to loading, 2 µL of 10X Orange-G loading dye in glycerol was added to each sample. The gel was electrophoresed at 80 V for 40 min and imaged in a Syngene G:BOX Gel Documentation System.

### 1.8. Cell line and growth conditions

Human embryonic kidney cells (HEK-293) were purchased from American Type Culture Collection (ATCC). The cells were grown in DMEM (Sigma, UK) supplemented containing 10% FBS and 0.5% Pen-strep and cultured in a humidified environment at 37 °C with 5% $CO_2$.

### 1.9. Confocal microscopy

Confocal microscopy images were obtained on an Axio Examiner Z1 high resolution laser scanning confocal microscope equipped with a Zeiss LSM780 scanhead in upright configuration. 405, 488, 561 and 640 nm lasers were used excite Hoechst 33342, EGFP, CX-Rhodamine and the deep red cell mask, respectively. An 10x EC objective (Plan-NeoFluar 10x/0.30) was used for image collection. HEK-293 cells were seeded in uncoated 35 mm MatTek glass bottom dishes (P35G-1.5-14-C) $3.0\times10^5$ cells/well in 2 mL growth media. After 24 h at 37 °C and 5% $CO_2$ in an incubator, the media was replaced with 1 mL growth media containing pDNA@NPs. From a 1.00 mg/mL NP stock solution 15 µL, 37.5 µL and 75 µL (corresponding to WR6, 15 and 30) were diluted to 75 µL with Milli-Q water and incubated with 2500 ng of EGFP pDNA for 15 min. After incubation, the suspension was mixed with 925 µL DMEM (10 % FBS) and administered to the cell culture. As positive control, Lipofectamine 2000 was prepared according to the manufacturer's instructions. After 24 h treatment, the medium was removed, and the cells were washed with 2 mL DPBS (2x). Hoechst 33342 nucleus stain and CellMask Deep Red plasma membrane stain according to the manufacturers protocol. Finally, the cells were mounted using ProLong™ Diamond Antifade Mountant and imaged.

### 1.10. EGFP pDNA-Labelling with CX-Rhodamine

EGFP pDNA was labelled with CX-Rhodamine using the LabelIT® Nucleic Acid Labelling Kit MIR3215 (MirusBio), following the manufacturer's protocol.[1] HEK-293 cells were seeded in MatTek dishes $3.0\times10^5$ cells/well in 2 mL growth media and incubated for 24 h. 15 µL, 37.5 µL and 75 µL (corresponding to WR6, 15 and 30) of a 1.00 mg/mL polyR-PDA NPs stock solution were diluted to 75 µL with Milli-Q water and incubated with 2500 ng of CX-Rhodamine-labelled pDNA for 15 min. The suspension was mixed with 925 µL DMEM (10 % FBS) and administered to the cell culture. For CX-Rhodamine-labelled pDNA tracking, the cultures were stained with Hoechst 33342 nucleus stain and CellMask Deep Red plasma membrane stain according to the manufacturers protocol. After staining, the cells were washed with 2 mL DPBS (2x). Finally, the cells were mounted using ProLong™ Diamond Antifade Mountant and imaged on an Axio Examiner Z1 high resolution laser scanning confocal microscope equipped with a Zeiss LSM780 scanhead as described in the confocal microscopy section.

### 1.11. Flow cytometry analysis

The cells were seeded in 6-well plates at a density of $3.0\times10^5$ cells per well in 2 mL culture media and cultured for 24 h. After 24 h the cells were treated with different pDNA@NPs WR6, WR15 and WR30 corresponding to 15, 37.5 and 75

µg/mL. Briefly, 15 µL, 37.5 µL and 75 µL were diluted to 75 µL with Milli-Q water and incubated with 2500 ng of EGFP pDNA for 15 min. The suspension was mixed with 925 µL DMEM (10 % FBS) and administered to the cell culture. As positive control, Lipofectamine 2000 was prepared according to the manufacturer's instructions. After 24 h treatment, cells were washed with PBS, detached with 0.25 mL TrypLE (Thermo Fischer, UK) and resuspended with FACS buffer (DPBS with 4% FBS). The cell suspensions were centrifuged for 15 min at 300*g*. 1 mL of FACS buffer (DPBS with 4% FBS) was added to the cells and containing 10 $\mu g mL^{-1}$ DAPI. The cells were kept at 4 °C until flow cytometry analysis. Flow cytometry was carried out on an CyAn ADP flow cytometer (Beckman Coulter) equipped with 405, 488 and 635 nm lasers in standard configuration. 100 000 events were acquired for each sample. FlowJo software (version 10.2) was used for data analysis. Briefly, the live single-cell population was gated in a plot of FSC *vs.* SSC after excluding cell debris and doublets a histogram from the FITC channel for the single-cell population was obtained and analysed.

### 1.12. Live cell transfection studies

HEK-293 cells were seeded in black 96-well plates (Corning, #3904) at a concentration of $2.0\times10^4$ cells/well in 100 µl of complete growth medium and incubated at 37 °C, 5% $CO_2$ for 24 h. Varying amounts of 1.00 mg/mL NPs stock solution (1.5, 3.7, 5.0, 7.5, 10.0 and 12.5 µL) corresponding to WR6, WR15, WR20, WR30, WR40 and WR50 were diluted with Milli-Q water to a final volume of 12.5 µL and incubated with 250 ng pDNA. After 15 min incubation DMEM (10% FBS) 50 µL was added to the 96-well plates. The plates were then inserted into the IncuCyte®S3 Live Cell Analysis System (Sartorius) for real-time imaging. Treated plates were imaged every hour for 48 h under cell culture conditions with a 20× objective using the green and brightfield channel. The mean fluorescence intensity was taken from 4 random fields of view per well and calculated with the IncuCyte S3 v2017A software.

### 1.13. MTS cytotoxicity studies

Cells were seeded into clear 96-well plates containing $2.0\times10^4$ cells per well in 100 µL complete growth medium and cultured for 24 h at 37 °C and 5% $CO_2$. Subsequently, cells were treated with varying concentrations of pArg, pHis-pArg-PDA NPs (15.0, 37.5 and 75.0 mg/mL), corresponding to WR6, WR15 and WR30. Briefly, 1.5 µL, 3.75 µL and 7.5 µL were diluted to 7.5 µL with Milli-Q water. Samples containing pDNA were incubated with 2500 ng of EGFP pDNA for 15 min. All samples were mixed with 92.5 µL DMEM (10 % FBS) and administered to the cell culture. As positive control, Lipofectamine 2000 was prepared according to the manufacturer's instructions. After further 24 h incubation at 37 °C and 5% $CO_2$, 20 µL of CelTiter 96® $AQ_{ueous}$ One Solution (Promega, USA) was added into each well and incubated at 37 °C, 5% $CO_2$ for 1–4 h, according to the manufacturer's instruction. The absorbance of each well was measured at 490 nm using a Spark plate reader (TECAN, CH). Control measurements included negative control of cells with DMEM containing 7.5% water (control), cell-free culture media (blank) and cell-free sample dilutions in culture media to evaluate potential sample interferences with MTS reagent. All experiments were conducted in biological triplicates. The percentage cell viability was calculated according to the following:

Cell viability (%) = 100x {[(Absorbance of treated cells) – (Absorbance of blank)] / [(Absorbance of control) – (Absorbance of blank)]}

## 2. Nanoparticle characterisation

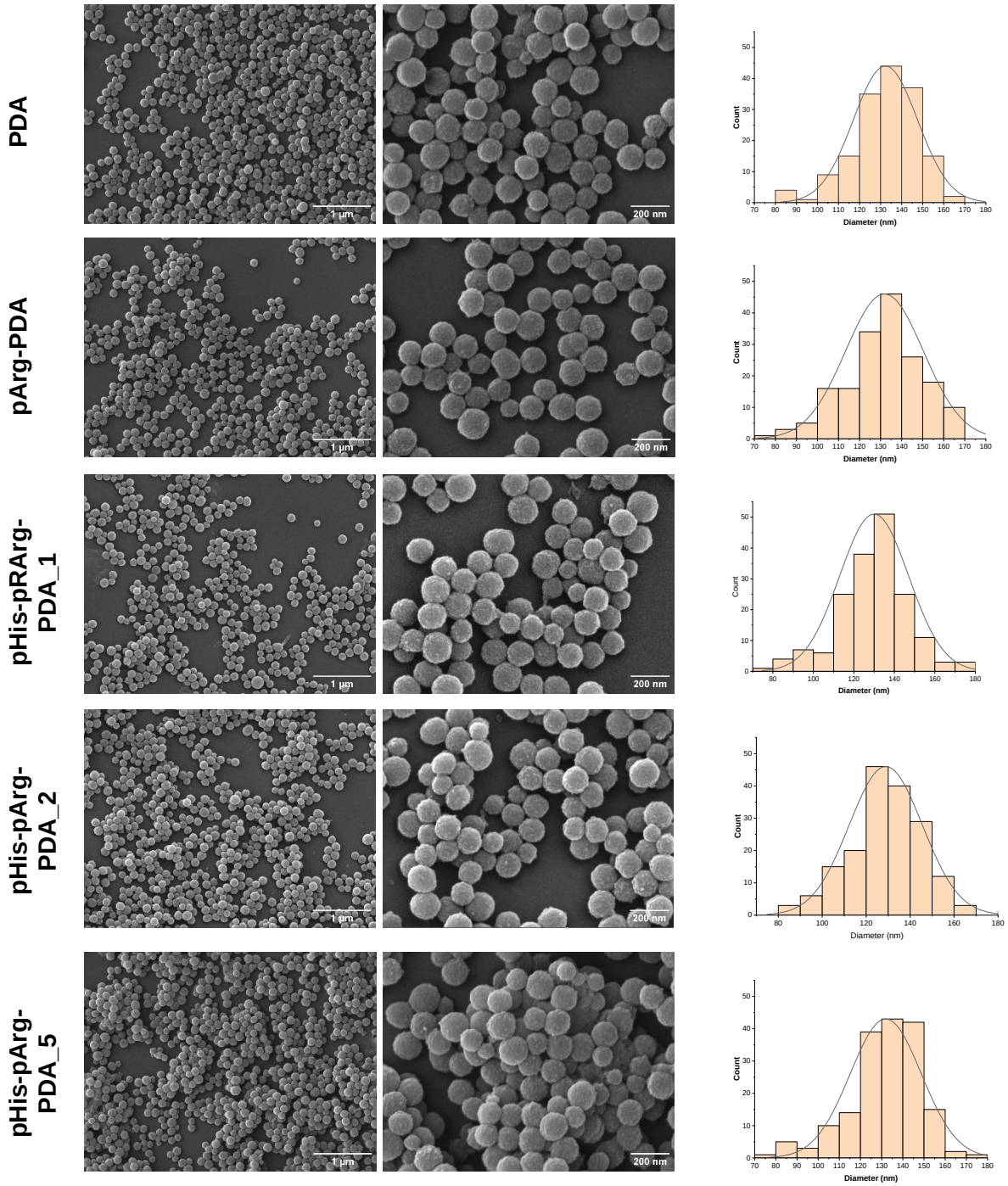


**Figure S1.** SEM micrographs of PDA, pR-PDA, pHpR-PDA_1, pHpR-PDA_2 and pHpR-PDA_5 and the size distribution obtained from the SEM images for N=175.

**Table S2.** Hydrodynamic size, polydispersity index (PDI) and zeta potential of PDA, pArg-PDA, pHis-pArg-PDA_1, pHis-pArg-PDA_2 and pHis-parg-PDA_5, as well as the average diameter of all NPs obtained from SEM images from (N=175).

| Sample | Hydrodynamic diameter [nm] | PDI | Zeta potential [mV] | SEM diameter [nm] |
|---|---|---|---|---|
| **PDA** | 158.4±39.2 | 0.065±0.03 | -29.0±7.1 | 135.9±13.4 |
| **pR-PDA** | 187.6±45.3 | 0.044±0.04 | +51.6±11.7 | 134.1±17.1 |
| **pHis-pArg-PDA_1** | 198.0±60.8 | 0.165±0.02 | +39.1±7.2 | 130.8±13.3 |
| **pHis-pArg-PDA_2** | 197.2±61.5 | 0.134±0.03 | +46.5±6.6 | 130.5±15.4 |
| **pHis-pArg-PDA_5** | 207.0±76.9 | 0.204±0.05 | +44.2±4.9 | 136.3±14.5 |

## 3. pDNA binding studies

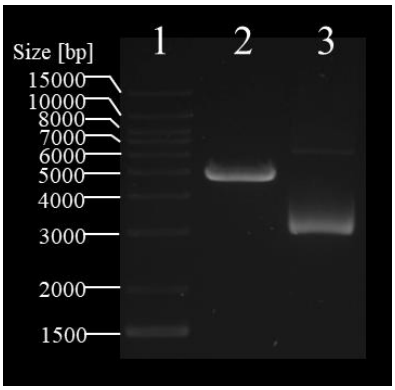


**Figure S2.** pDNA size analysis assay. Lane 1: 1 Kb plus DNA ladder (1500 – 1500bp), Lane 2: EGFP-pDNA (4731 bp) linearised with 10 units HindIII-HF restriction enzyme, Lane 3: Untreated pDNA. The gel was stained with SYBR Safe dye.

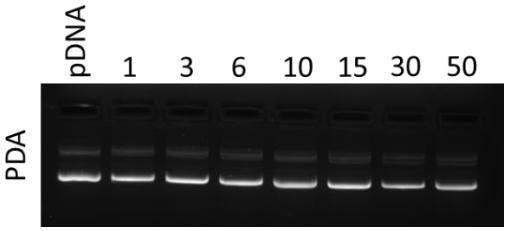


**Figure S3.** Agarose gel electrophoresis of PDA NP complexes with pDNA WR1 – WR50. The gel was stained with SYBR Safe dye.

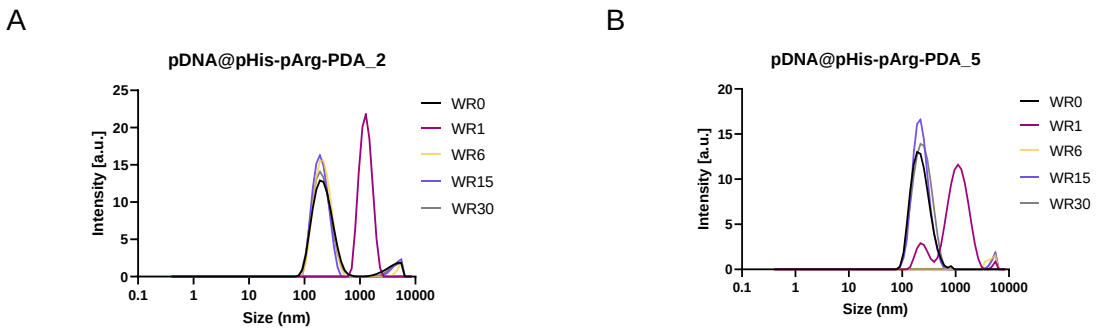


**Figure S4.** Hydrodynamic size pDNA@pHis-pArg-PDA_2 (A) and pDNA@pHis-pArg-PDA_5 (B) measured for WR0, WR1, WR6, WR15 and WR30.

**Table S3.** Hydrodynamic size, PDI and zeta potential of PDA, pArg-PDA, pHis-pArg-PDA_1, pHis-pArg-PDA_2 and pHis-pArg-PDA_5 NPs and their pDNA complexes at WR1, WR6. WR15 and WR30.

| Sample | Hydrodynamic diameter [nm] | PDI | Zeta potential [mV] |
|---|---|---|---|
| **pArg-PDA** | 188.4±46.4 | 0.014±0.012 | +53.8±11.7 |
| **pDNA@pArg-PDA (WR1)** | 945.1±53.7 | 0.540±0.310 | -32.3±5.4 |
| **pDNA@pArg-PDA (WR6)** | 365.5±149.6 | 0.157±0.011 | +22.8±10.0 |
| **pDNA@pArg-PDA (WR15)** | 185.6±39.59 | 0.013±0.006 | +41.6±11.3 |
| **pDNA@pArg-PDA (WR30)** | 187.5±41.12 | 0.046±0.015 | +54.1±7.6 |
| **pHis-pArg-PDA_1** | 208.2±62.3 | 0.233±0.037 | +38.5±6.9 |
| **pDNA@pHis-pArg-PDA_1 (WR1)** | 912.9±189.9 | 0.310±0.054 | -10.8±4.1 |
| **pDNA@pHis-pArg-PDA_1 (WR6)** | 273.3±71.2 | 0.298±0.029 | +22.1±6.2 |
| **pDNA@pHis-pArg-PDA_1 (WR15)** | 217.4±88.3 | 0.176±0.035 | +31.2±5.9 |
| **pDNA@pHis-pArg-PDA_1 (WR30)** | 215.9±75.5 | 0.270±0.033 | +36.0±6.4 |
| **pHis-pArg-PDA_2** | 216.4±77.9 | 0.273±0.012 | +44.2±4.9 |
| **pDNA@pHis-pArg-PDA_2 (WR1)** | 1256±282.4 | 0.460±0.048 | -8.04±3.3 |
| **pDNA@pHis-pArg-PDA_2 (WR6)** | 218.4±77.5 | 0.295±0.027 | +26.8±6.1 |
| **pDNA@pHis-pArg-PDA_2 (WR15)** | 202.5±62.9 | 0.267±0.019 | +35.8±5.8 |
| **pDNA@pHis-pArg-PDA_2 (WR30)** | 212.1±79.8 | 0.269±0.056 | +37.3±6.9 |
| **pHis-pArg-PDA_5** | 257.9±107.7 | 0.296±0.042 | +46.5±6.6 |
| **pDNA@pHis-pArg-PDA_5 (WR1)** | 1198.2±31.7 | 0.238±0.021 | -7.6±4.2 |
| **pDNA@pHis-pArg-PDA_5 (WR6)** | 231.3±96.5 | 0.279±0.014 | +27.4±7.5 |
| **pDNA@pHis-pArg-PDA_5 (WR15)** | 232.1±47.9.5 | 0.266±0.008 | +35.5±7.6 |
| **pDNA@pHis-pArg-PDA_5 (WR30)** | 258.9±102.4 | 0.259±0.031 | +37.3±10.5 |

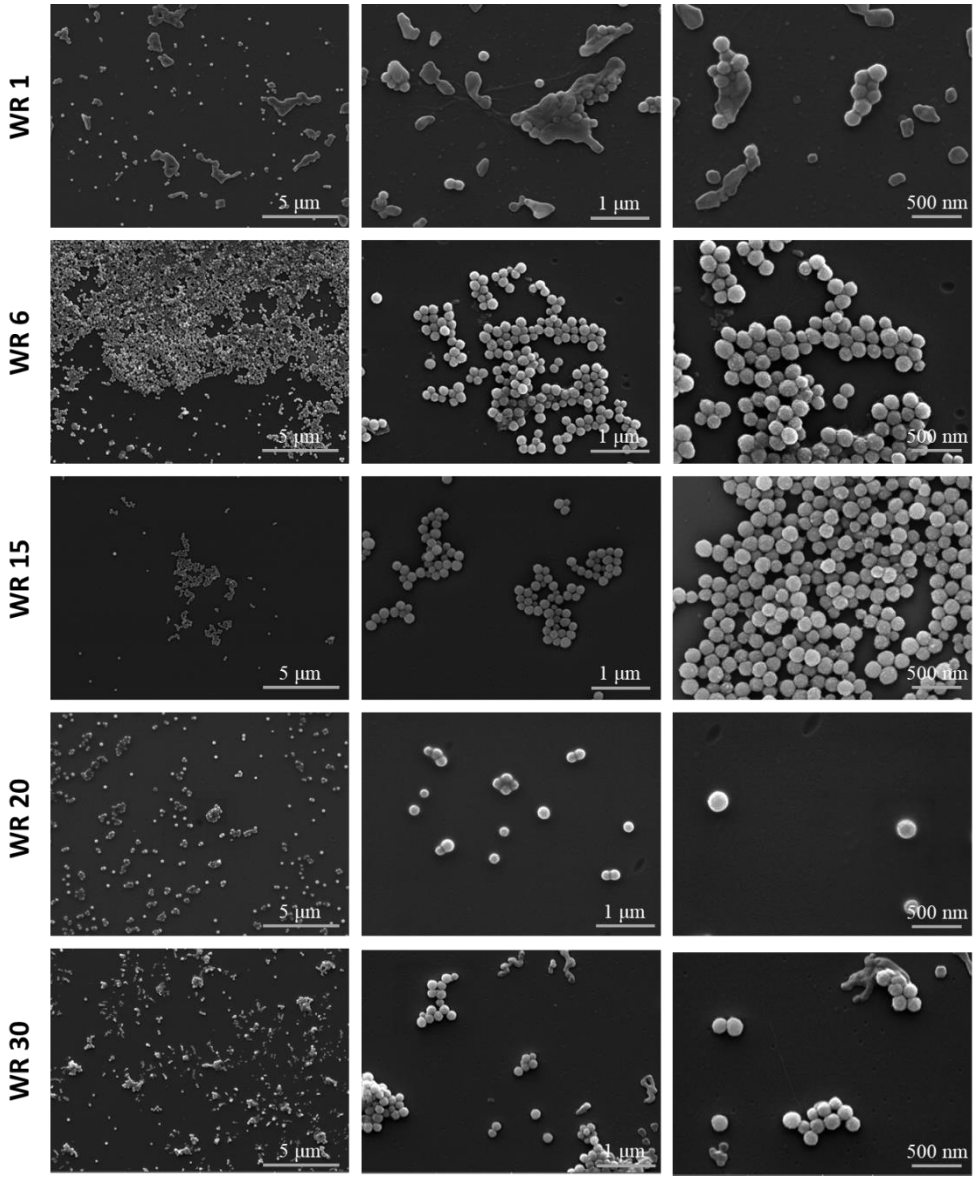


**Figure S5.** SEM Micrographs WR1, WR6, WR15, WR20 as well as WR30 pDNA@pArg-PDA NPs dispersed on glass slides and coated with 10 nm sputtered Cr. Micrographs 1-3 show three different locations on the glass slide for each sample respectively.

## 4. Confocal microscopy of pArg and pHis-pArg modified PDA NPs

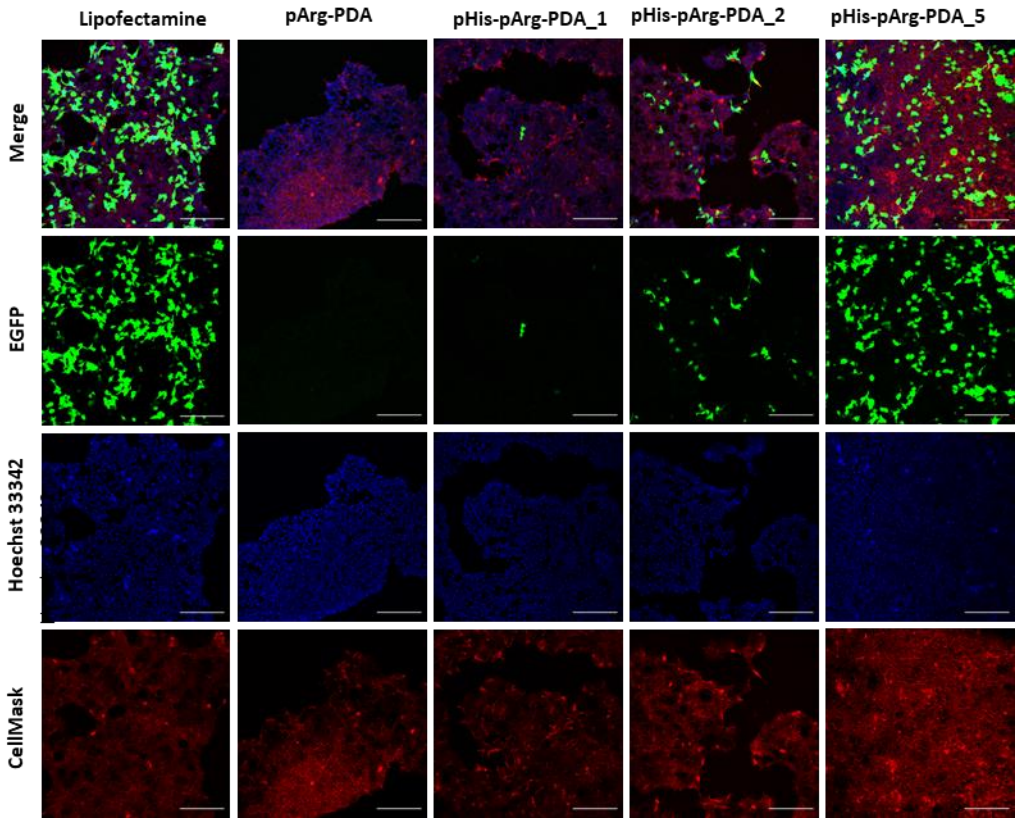


**Figure S6.** Confocal microscopy images of WR6 pDNA complexes with pArg-PDA, pHis-pArg-PDA_1, pHis-pArg-PDA_2 and pHis-pArg-PDA_5 NP formulations after 24 h administration to HEK-293 cells. Lipofectamine was used as a positive control. Cells were incubated with CellMask (deep red) and Hoechst 33342 (blue) to stain cell membrane and nuclei, respectively. Green channel captured the fluorescence of EGFP. The scale bar is 200 μm.

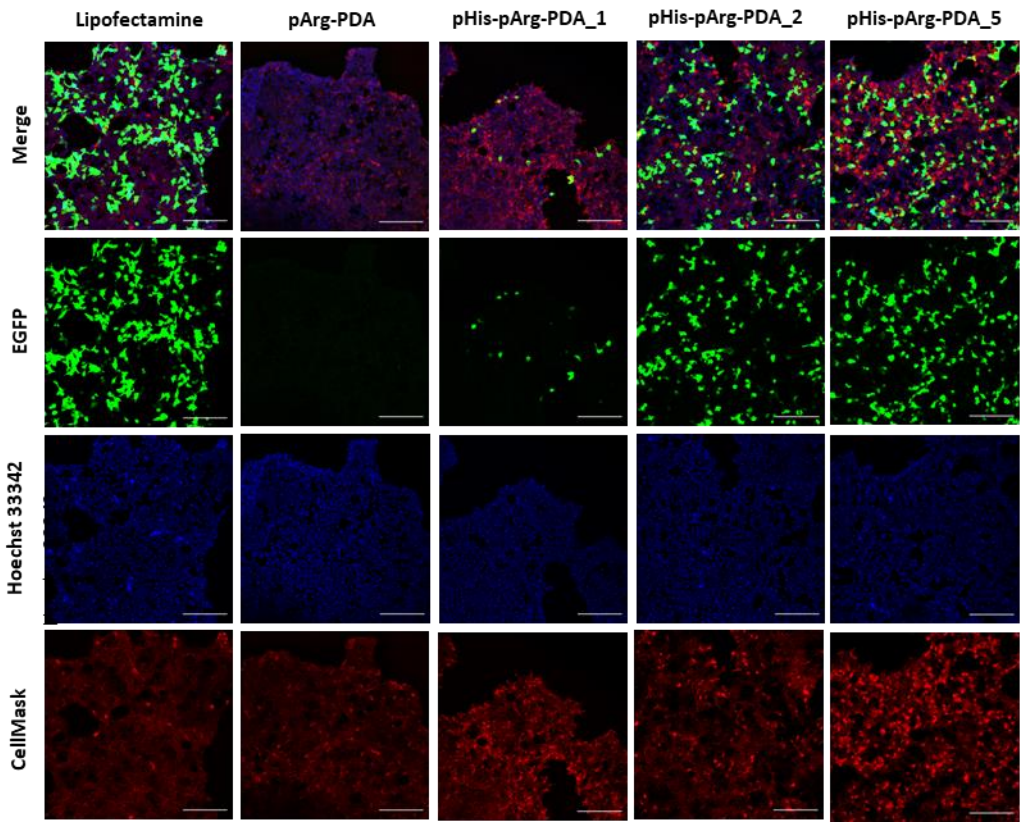


**Figure S7.** Confocal microscopy images of WR15 pDNA complexes with pArg-PDA, pHis-pArg-PDA_1, pHis-pArg-PDA_2 and pHis-pArg-PDA_5 NP formulations after 24 h administration to HEK-293 cells. Lipofectamine was used as a positive control. Cells were incubated with CellMask (deep red) and Hoechst 33342 (blue) to stain cell membrane and nuclei, respectively. Green channel captured the fluorescence of EGFP. The scale bar is 200 μm.

## 5. Confocal microscopy studies with CX-Rhodamine pDNA

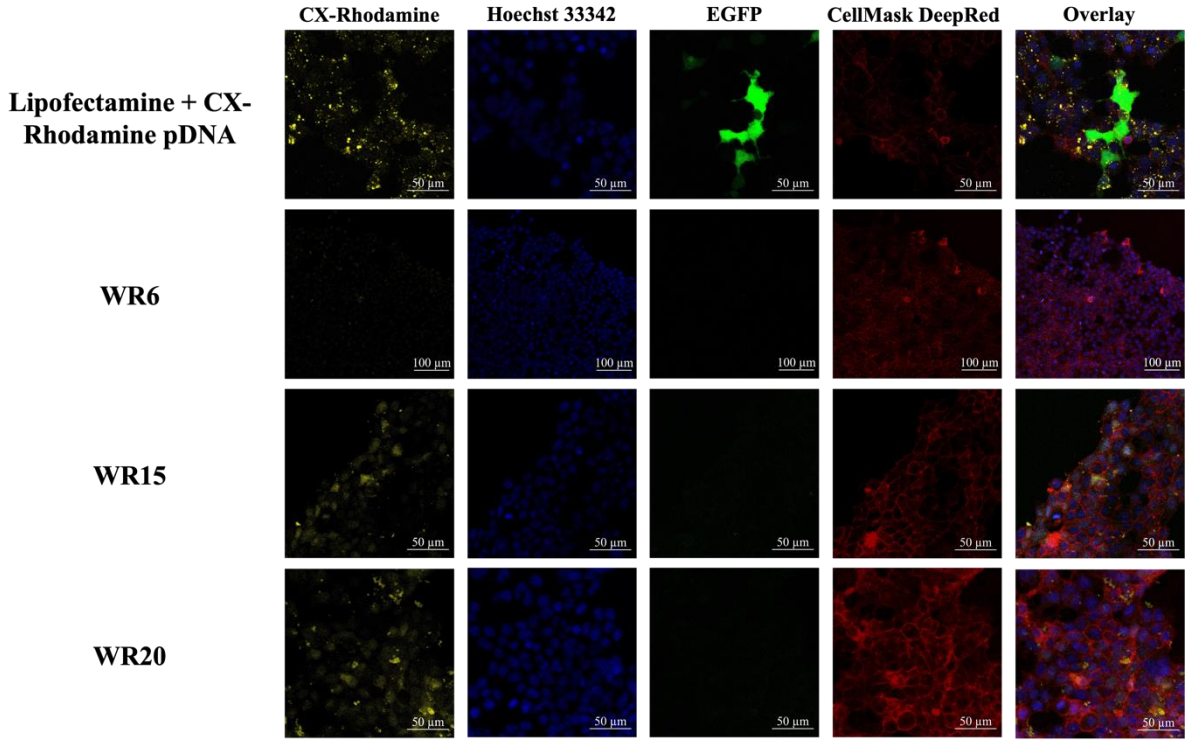


**Figure S8.** Confocal Microscopy transfection study CX-Rhodamine-labelled pDNA-carying pArg-PDA NPs (WR6, WR15 and WR20) post 24 h administration of formulations to HEK-293 cells. Lipofectamine/CX-Rhodamine-labelled pDNA complexes were administered as reference and positive control. Samples were stained with nucleus dye (Hoechst 33342) and plasma membrane dye (CellMask DeepRed). Micrographs are shown as overlay of Hoechst 33342, EGFP, CX-Rhodamine and DeepRed channels.

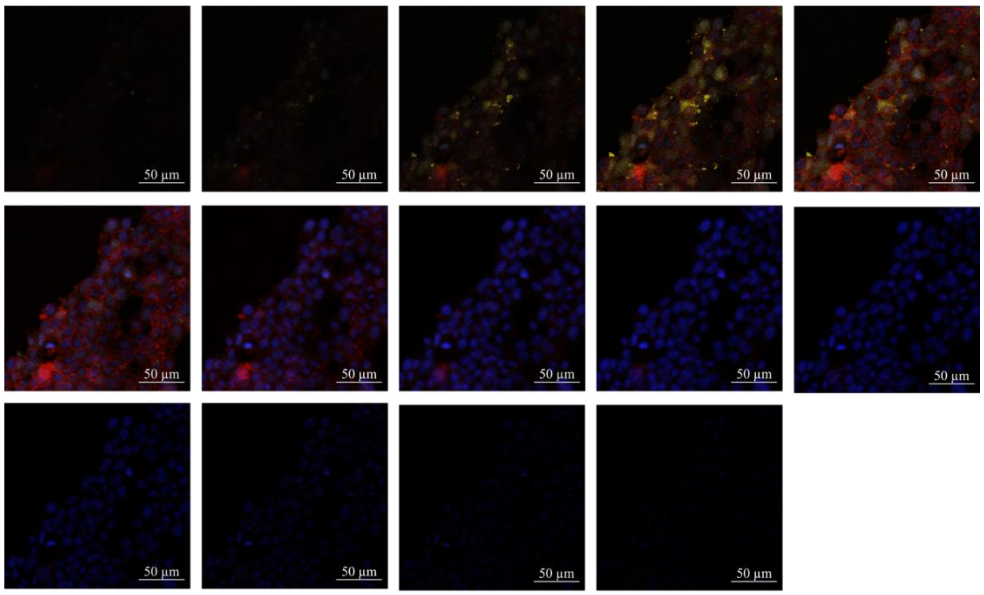


**Figure S9.** Confocal Microscopy z-stack series of CX-Rhodamine-labelled pDNA-carying pArg-PDA NPs WR15, post 24 h administration of the formulation to HEK-293 cells. Samples were stained with nucleus dye (Hoechst 33342) and plasma membrane dye (CellMask DeepRed). Micrographs are shown as overlay of Hoechst 33342, EGFP, CX-Rhodamine and DeepRed channels.

## 6. FACS analysis

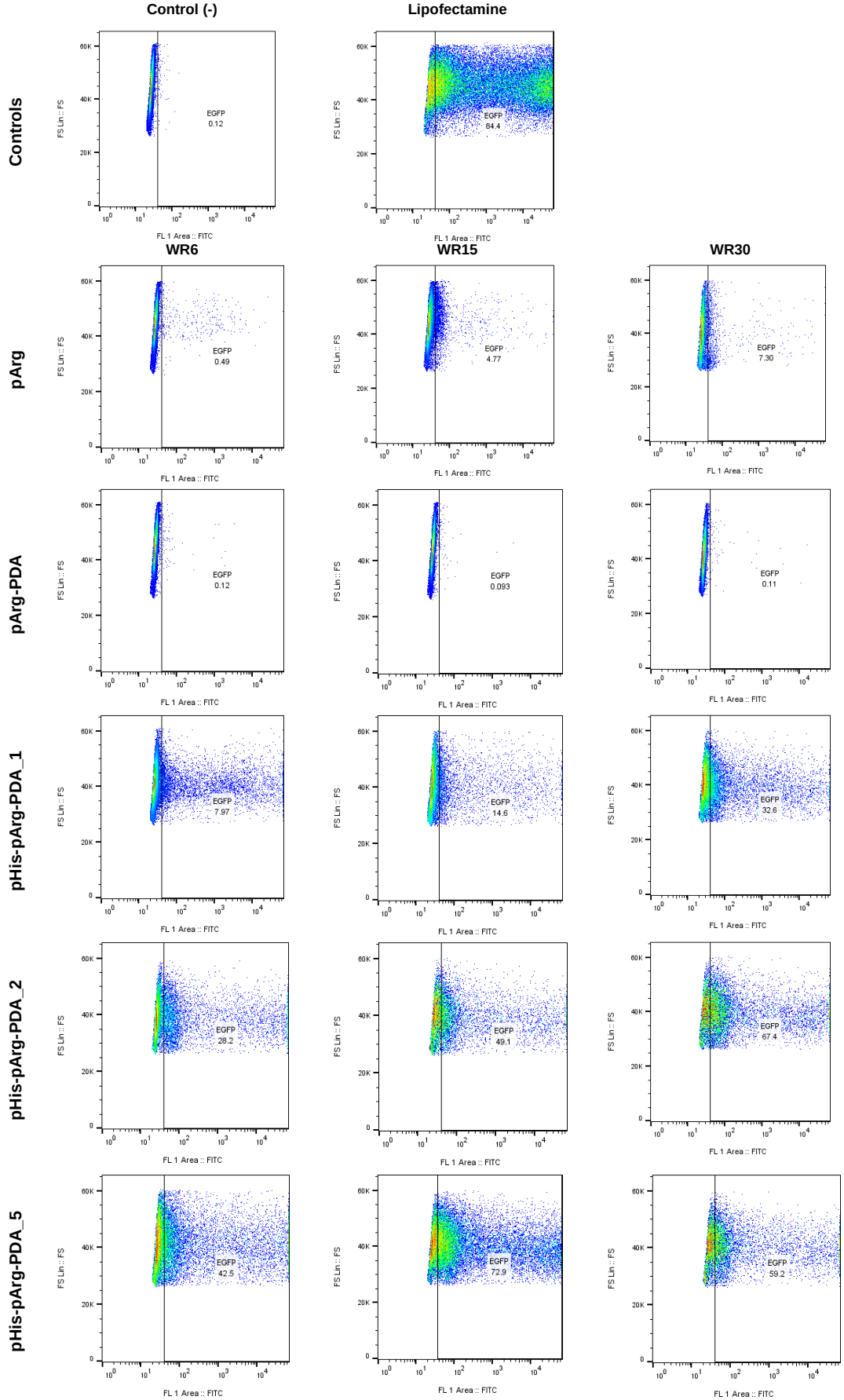


**Figure S10**. FACS analysis of EGFP expressing HEK-293 cells for pDNA complexes with pArg, pArg-PDA and pHis-pArg-PDA NP. Example of FITC vs FSC graphs after 24 h treatment with WR6, WR15 and WR30.

## 7. Cell viability

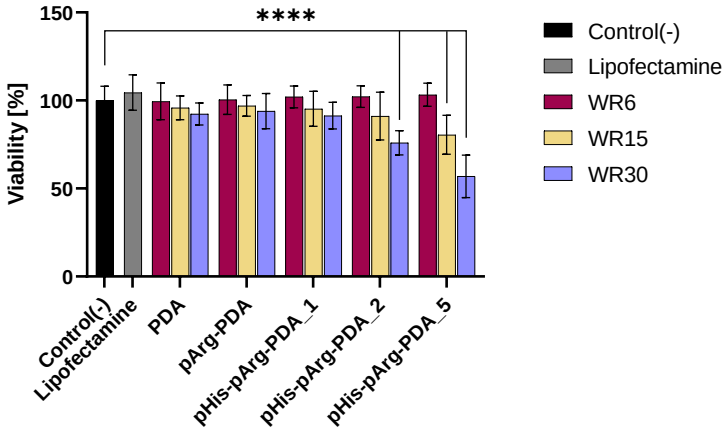


**Figure S11.** Viability of HEK-293 cells treated with PDA, pArg-PDA and pHis-pArg PDA NPS**.** The effect on cell viability was evaluated for all NPs at WR6, WR15 and WR30 corresponding to 15, 37.5 and 75 µg/mL NP concentrations after 24 h treatment using MTS assay. As controls the viability of unmodified PDA NPs and lipofectamine was also determined. Data are expressed as mean ± SD obtained from three separate measurements. One-way ANOVA was used to compare the viability to the untreated cells, Control(-). Significance levels are defined as the following: ns for $p > 0.05$, * for $p \leq 0.05$, ** for $p \leq 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$.

## 8. References

[1] P. S. Slattum, A. G. Loomis, K. J. Machnik, M. A. Watt, J. L. Duzeski, V. G. Budker, J. A. Wolff, J. E. Hagstrom, *Mol. Ther.* **2003**, *8*, 255–263.