Electronic Supplementary Information

Phenylboronic acid-functionalized polyaminoglycoside as one effective CRISPR/Cas9 delivery system

Meiyu Shao, Yu Qi, Dandan Sui* and Fu-Jian Xu*

Key Laboratory of Biomedical Materials of Natural Macromolecules (Beijing University of Chemical Technology), Ministry of Education, Beijing Laboratory of Biomedical Materials, College of Materials Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, China

* Correspondence and requests for materials should be addressed to F.-J.X. (email: xufj@mail.buct.edu.cn) or to D. S. (email: suidd@mail.buct.edu.cn).

Materials and animals. Tobramycin, triethylamine (TEA, 99.5%), 3-

aminophenylboronic acid monohydrate (APBA), ethylenediamine (ED) and branched polyethylenimine (PEI, 98%, Mw ~25 kDa) were purchased from Sigma-Aldrich (USA). Anhydrous dimethyl sulfoxide (DMSO) was purchased from Alfa Aesar (USA). Dithiothreitol (DTT, 99%) and methylthiazolyldiphenyl-tetrazolium bromide (MTT, 98%) were purchased from Energy & Chemical Co., Ltd. (Shanghai, China). Temozolomide (TMZ) was purchased from Tokyo Chemical Industry Co. Ltd. (Japan). Hanks' Balanced Slat Solution (pH 7.4), FITC-lectin conjugates (FITC-MAL and FITC-SNL) were purchased from VECTOR Co., Ltd. (USA). HEK 293 and A549 cell lines were purchased from American Type Culture Collection (ATCC, Rockville, MD). Plasmid pCas9-survivin which encodes Cas9 and GFP as well as transcribes survivin sgRNA (5'-TCTTGAATGTAGAGATGCGG-3') was purchased from Nanjing GenScript Biotechbology Co. Ltd. (China). Reporter plasmid (pRL-CMV, encoding renilla luciferase) and plasmid pCas9-survivin were amplified in E. coli and purified according to the supplier's protocol (Omega Bio-tek, Inc., USA). Renilla Luciferase Assay Kit (Promega Co., Cergy Pontoise, France), YOYO^{TM}-1 staining reagent, 4',6-diamidino-2-phenylindole (DAPI), Protein Extracted Kit, lgG-HRP and Cyanine7-N-hydroxysuccinimide ester (Cy7-NHS) were obtained from Beijing Solarbio Science & Technology Co., Ltd. (China). Taq PCR MasterMix (2×) and T7 Endonuclease I (T7EI) were purchased from Cell Biolabs, Inc. (USA). Anti-survivin antibody and anti-β-Tubulin antibody were purchased from Abcam Co., Ltd. (USA). Nude BALB/c mice (female, 6-week-old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (China).

Physical characterization of materials. The chemical structures and molecular weights of synthesized SS-HPT-based polymers were characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy (Bruker ARX 400 MHz spectrometer) and gel permeation chromatography (GPC), respectively. For agarose gel electrophoresis assay, the polycation (SS-HPT, SS-HPT-P1 and SS-HPT-P2)/pDNA (plasmid DNA) complexes were formed by mixing appropriate volumes of polymer solution (0.1 mg/mL in DI water) and pDNA solution (0.1 mg/mL in DI water) at w/w ratios of 0-4. It was conducted by using a Sub-Cell system (Bio-Rad Laboratory, Hercules, CA) to

measure the ability of polycations to retard pDNA. A Zetasizer Nano ZS system (Zetasizer Nano ZS90, UK) was utilized to determine the particle sizes and zeta potentials of nanoparticles at different stages. The visualized images of nanoparticles were captured using AFM equipped with a Nanoscope IIIa controller (Bruker Dimension Icon).

Cell culture. HEK 293 and A549 cell lines were used to evaluate the cytotoxicity of polycations. The cells were cultured in dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS, provided by Gibco), 1% penicillin and 1% streptomycin. The incubation environment requires a 5% CO_2 atmosphere and 95% relative humidity with a temperature set at 37 ˚C.

Intracellular uptake assay. 8×10^5 A549 cells in each well were seeded into 6-well plates and cultured for 24 h. Report plasmid pDNA was labeled with fluorescent dye YOYO-1 in dark for 2 h beforehand and then mixed with polycations for 30 min. The PEI/pDNA, SS-HPT/pDNA and SS-HPT-P2/pDNA complexes (containing 6 μg of labeled pDNA) were added to each well. After 4 h of co-culture, the cells were washed with PBS for 3 times. The cultured cells were labeled by DAPI for 10 min and imaged under a Leica DMI3000 B Fluorescence Microscope. The percentage of positive cells was detected by flow cytometry.

Statistical analysis. All experiments were repeated at least three times for statistical analysis and the data were presented as means ± standard deviation. One-way ANOVA with Bonferroni's correction was used to compare differences between at least three groups, and Student's t-test for two groups. In all pictures, $P^* < 0.05$ represented one star, $P^{**} < 0.01$ represented two stars.

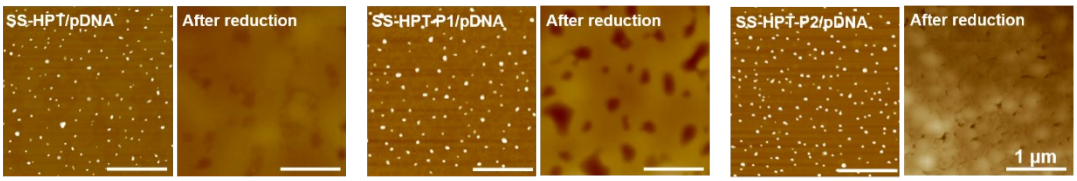

Figure S1. Representative AFM images of SS-HPT/pDNA, SS-HPT-P1/pDNA and SS-HPT-P2/pDNA complexes before or after degradation.

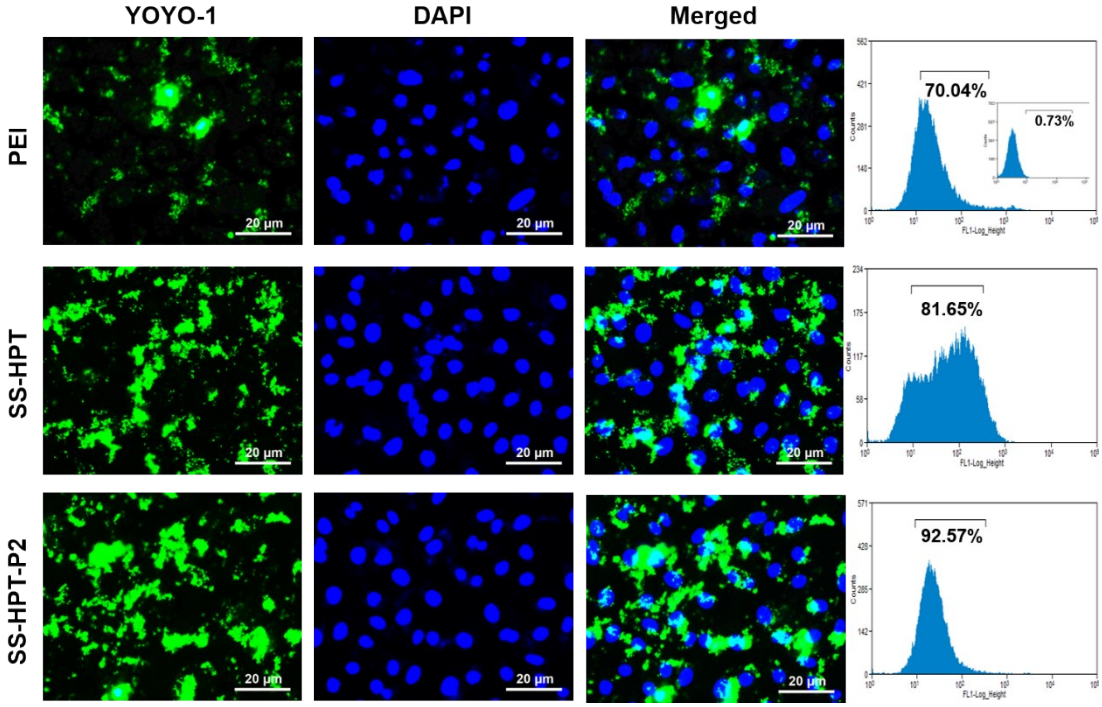

Figure S2. Fluorescence microscope images and flow cytometry analysis of A549 cells treated with PEI/pDNA, SS-HPT/pDNA, and SS-HPT-P2/pDNA complexes (YOYO-1-labeled pDNA) at their optimal N/P and w/w ratios.

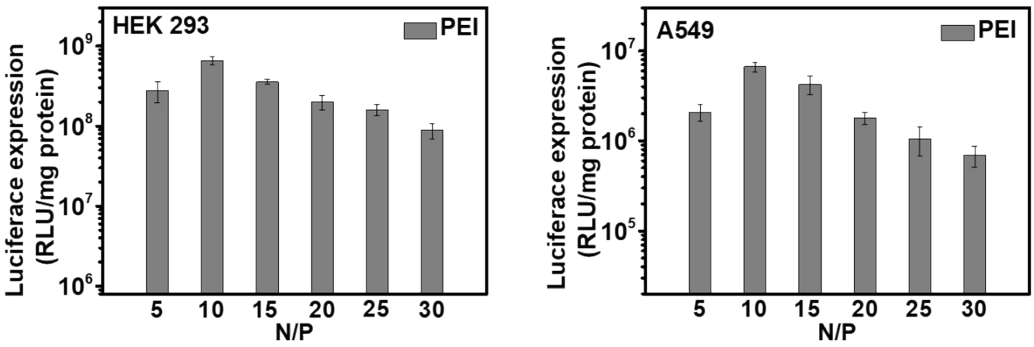

Figure S3. Luciferase expression of HEK 293 and A549 cell lines treated by PEI/pDNA complex at various N/P ratios.

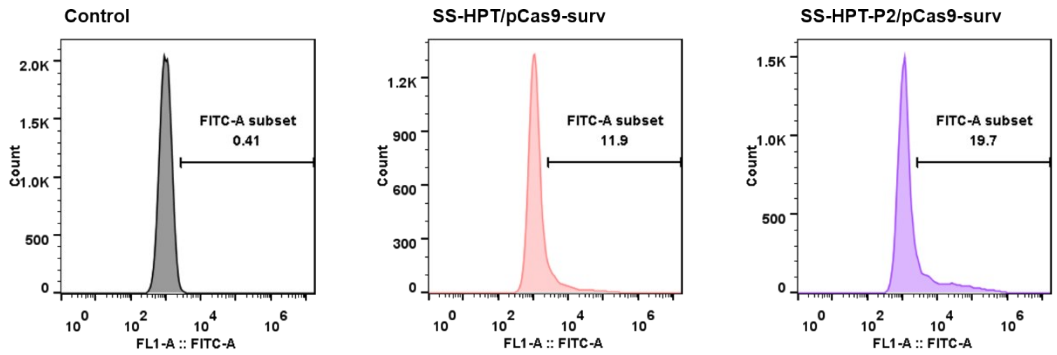

Figure S4. Flow cytometry analysis of GFP expression treated with SS-HPT/pCas9-surv and SS-HPT-P2/pCas9-surv complexes in A549 cell line after 24 h transfection.

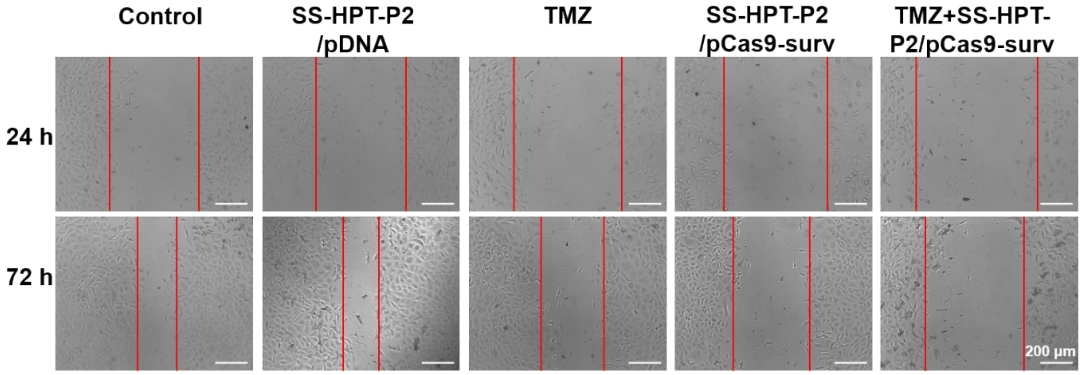

Figure S5. Representative images of cell migration of A549 cells in different treatment groups at 24 and 72 h.