Support Informations

Bioreducible Poly(Urethane Amine)s for Robust Nucleic Acid Transfection in Stem Cells

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Table S1. Molecular weight and polydispersity index (PDI) of polyurethanes

Polymers	M_w (kDa) [a]	PDI [a]
SSBAP	19.6	1.6
SSBT10	16.8	1.8
SSBT30	31.6	1.6
SSBT60	36.3	1.5
SSMDE	15.8	1.5
SSPDA	11.3	1.6

[a] Weight average molecular weight (M_w) is determined by GPC analysis

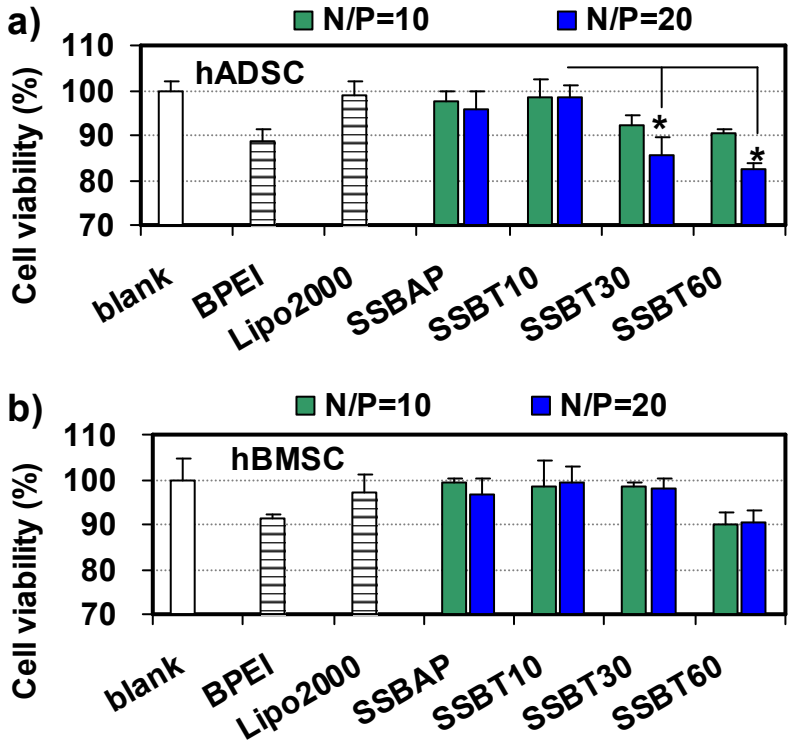

Figure S1. Alamar blue assay showing cytotoxicity of SSBAP and SSBT copolymers against human stem cells after transfection using a serum-free transfection protocol. * $P<0.05$.

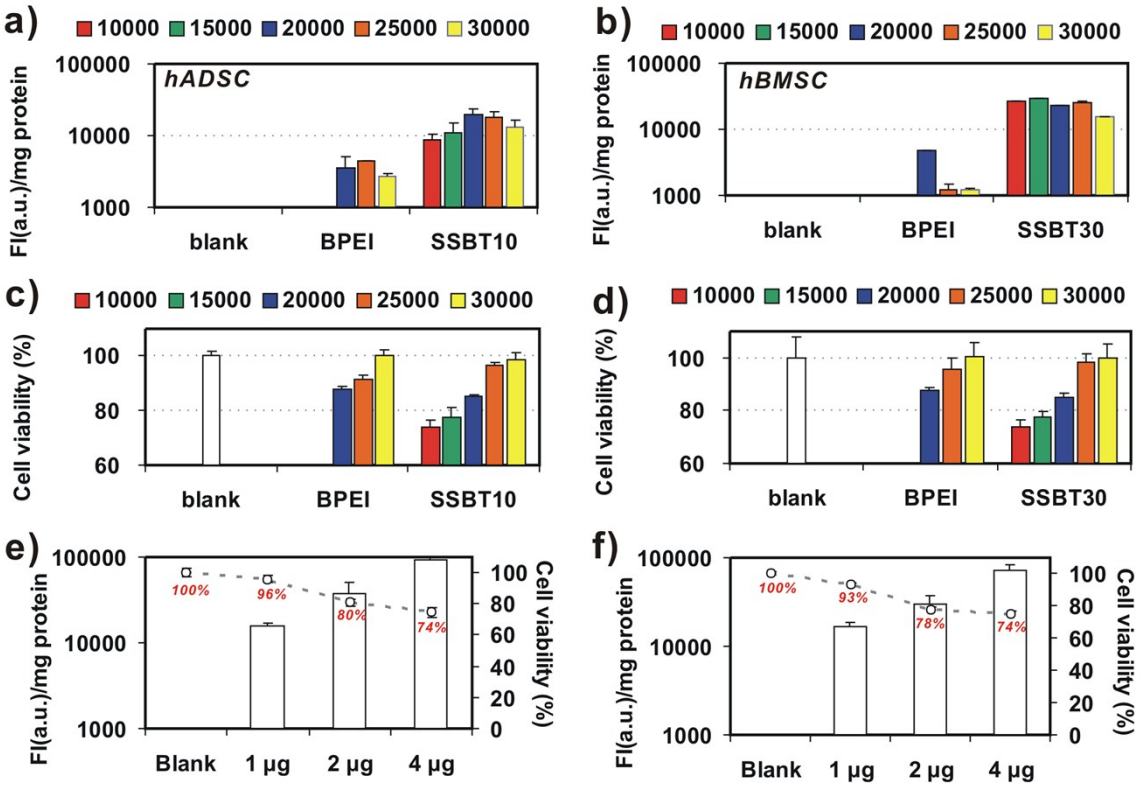

Figure S2. Effect of cell seeding density (10000-30000 cells/cm^2) on transfection activity and cytotoxicity of SSBT polyplexes (N/P=20/1) against human stem cells using a serum-free transfection protocol. a-b) GFP expression level induced by a) SSBT10 polyplexes against hADSCs and b) SSBT30 polyplexes against hBMSCs at an N/P ratios of 20/1; c-d) cytotoxicity of c) SSBT10 polyplexes against hADSCs and d) SSBT30 polyplexes against hBMSCs; e-f) At a cell density of 30000 cells/cm^2, GFP expression level and cell viability induced by e) SSBT10 polyplexes against hADSCs and f) SSBT30 against hBMSCs as a function of DNA dose (1-4 μg). BPEI polyplexes (N/P=10/1) were used as a control.

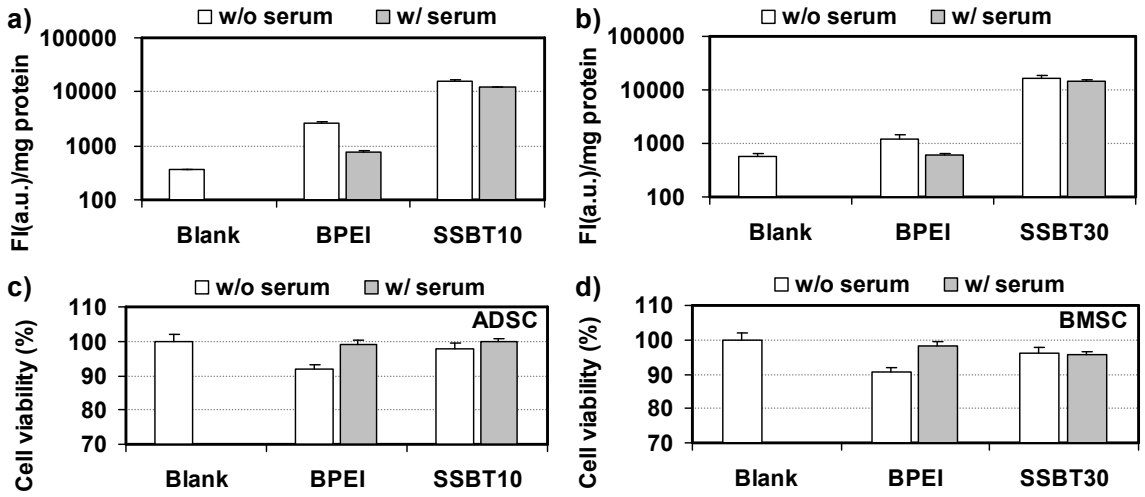

Figure S3. Transfection efficiency and cytotoxicity of SSBT polyplexes (N/P=20/1) against human stem cells using a serum-free (w/o) or a serum-containing (w/) transfection protocol. a-b) GFP expression level afforded by a) SSBT10 polyplexes against hADSCs and b) SSBT30 polyplexes against hBMSCs; c-d) cytotoxicity of the polyplexes against c) hADSCs and d) hBMSCs. BPEI polyplexes (N/P=10/1) were used as a control.

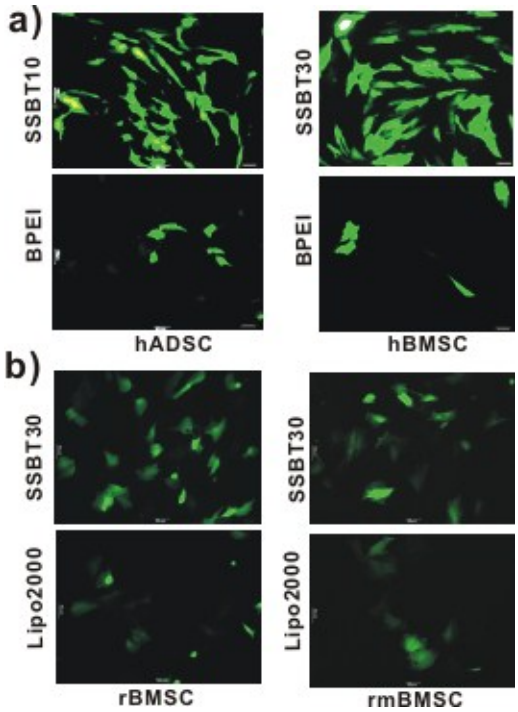

Figure S4. Fluorescence imaging of GFP-expressing a) human stem cells and b) animal stem cells induced by SSBT10 or SSBT30 using a serum-free transfection protocol. BPEI or Lipofectamine 2000 (Lipo 2000) was used as a positive control.

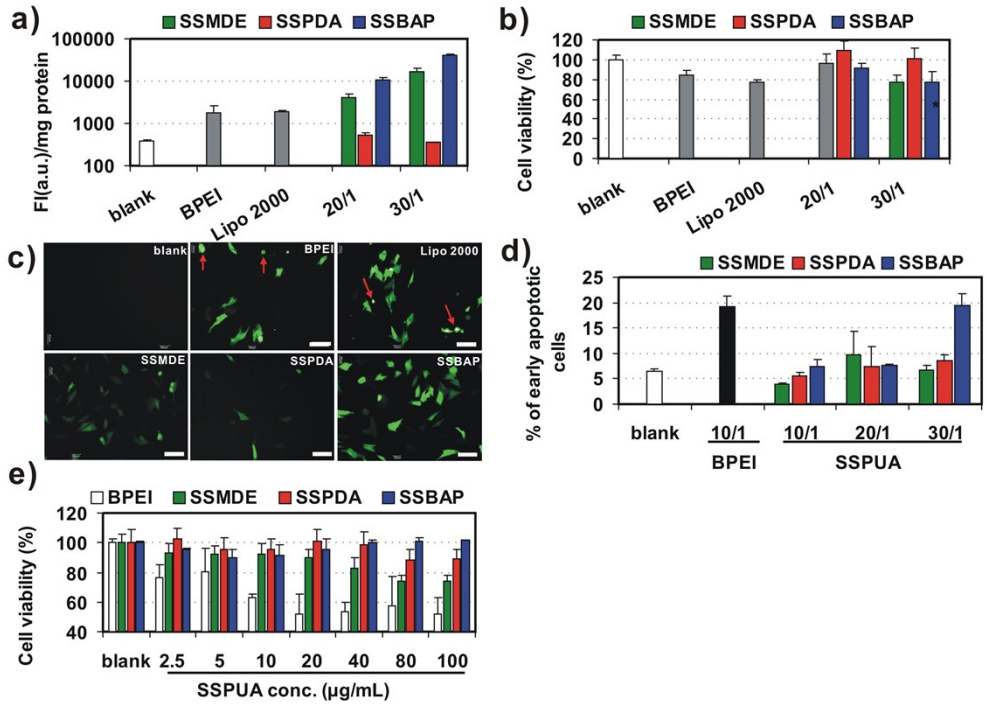

Figure S5. Transfection activity of SSPUAs against canine BMSCs (cBMSCs) using a serum-free transfection protocol. a) GFP expression level and b) cytotoxicity of SSPUA polyplexes at N/P ratios; c) Typical fluorescence images of GFP-expressing cBMSCs induced by SSPUA-based polyplexes at an N/P ratio of 20/1(scale bar: 50 μm). Red arrow indicates the MSCs in a round shape; d) Annexin V-FITC/PI staining assay showing percentage of early apoptotic cBMSCs after cBMSCs were transfected by SSPUA polyplexes at N/P ratios. BPEI polyplexes (N/P=10/1) was used as a control; e) AlamarBlue assay showing the cytotoxicity of SSPUAs or BPEI against cBMSCs at varied polymer concentrations.

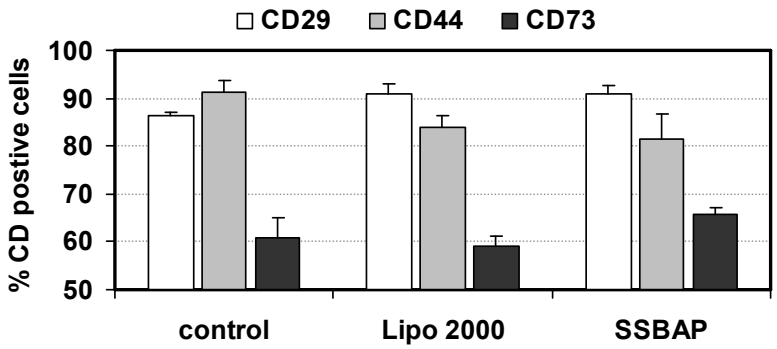

Figure S6. Flow cytometry showing the percentages of stemness marker (CD29, 44, 73)-positive rmBMSCs after rmBMSCs were transfected by Lipofectamine 2000 (Lipo 2000) or SSBAP using a serum-free transfection protocol. Untreated cells were used as a control.

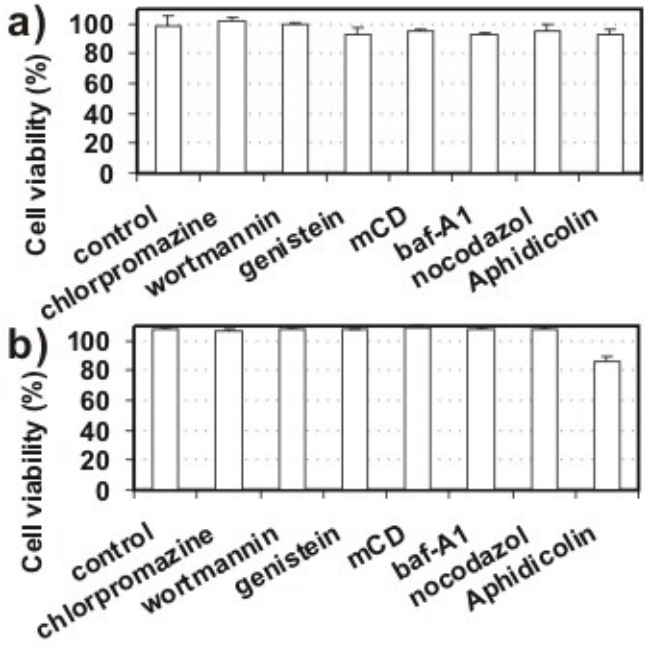

Figure S7. Cytotoxicity of a) SSBT10 polyplexes against hADASCs and b) SSBT30 polyplexes against hBMSCs using a serum-free transfection protocol after the cells were treated by different inhibitors and then transfected by the polyplexes (N/P=20/1).

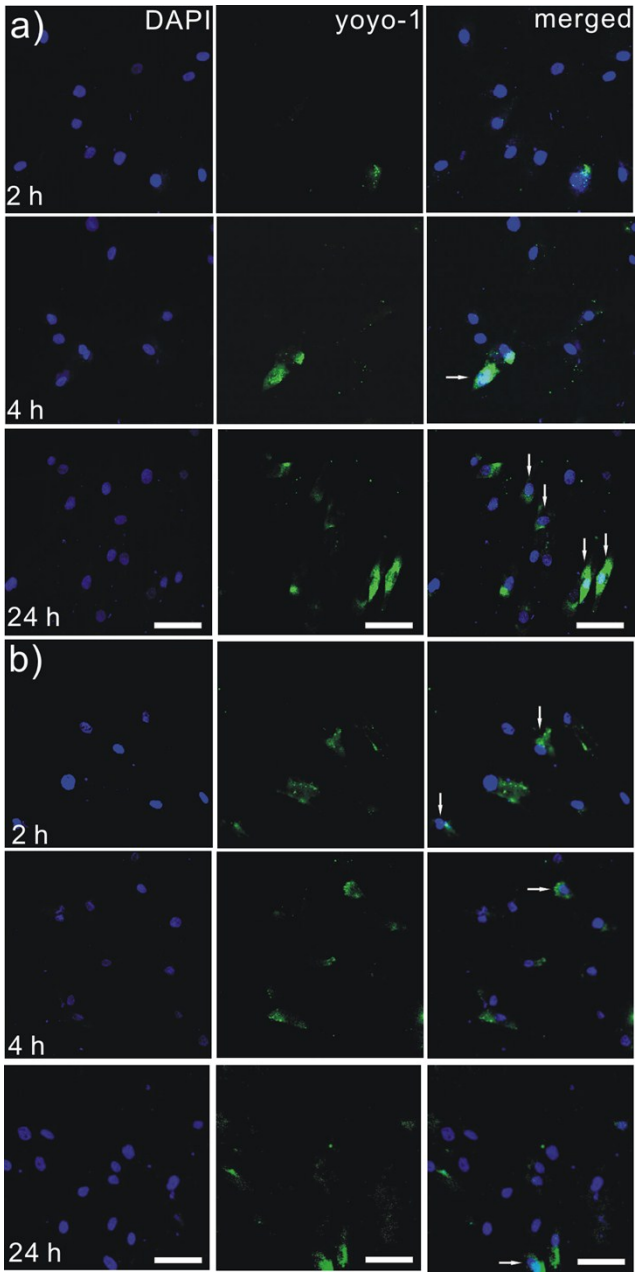

Fig.S8. Confocal laser microscope showing the location of yoyo-1-labeled DNA (green) in a) hADSCs and b) hBMSCs at different transfection time (2, 4 and 24 h) after the cells were transfection by SSBT10 and SSBT30 polyplexes, respectively, at an N/P ratio of 20/1, using a serum-free transfection protocol. The white arrow shows that DNA was found in the nucleus (stained by DAPI) of hADSCs at 4 h-post transfection time but earlier in the nucleus of BMSCs at 2 h-post transfection time. Scale bar: 100 μm.

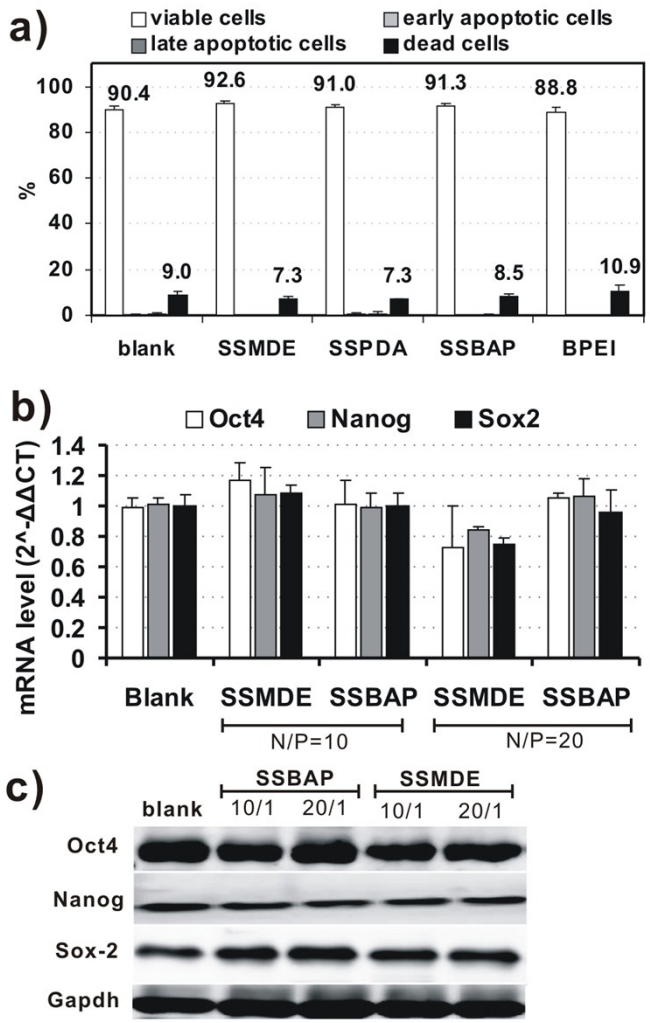

Fig. S9. Transfection activity of SSPUAs against mESCs using a serum-containing transfection protocol. a) Annexin V-FITC/PI staining showing the percentages of viable and early/late apoptotic mESCs after treated by SSPUA polyplexes (N/P=20/1); e) Effect of SSPUA polyplexes at N/P ratios on mRNA expression levels of three mESC pluripotency markers (*i.e.* Nanog, Oct4 and Sox-2) using qRT-PCR assay. mRNA expression levels was calculated by Pfaffl method of $2^{-(\Delta\Delta CT)}$. The levels was normalized to those of untreated cells as the blank (taken as 1.0). c) Western blotting assay showing protein expression of mESC pluripotency markers after the transfection by SSPUA polyplexes at different N/P ratios. The housekeeping gene Gapdh was used as an internal control. All values are given as the mean±SD (n=3).

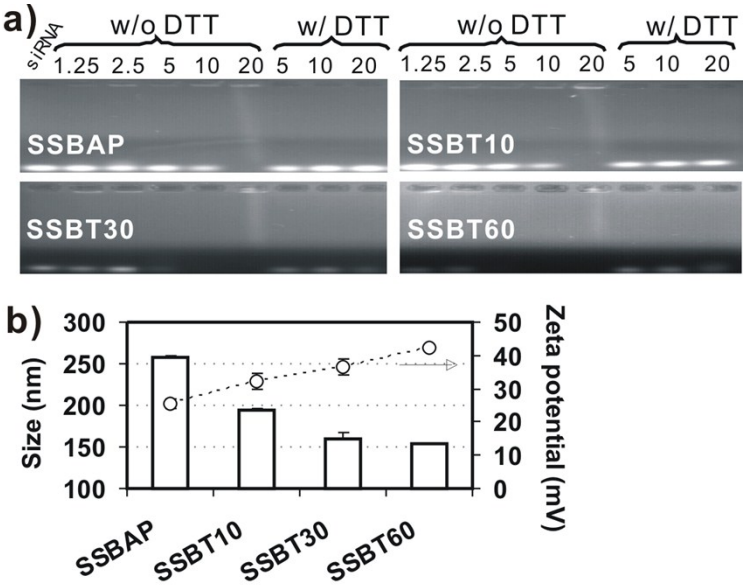

Fig. S10. a) Gel retardation assay of SSPUA/siRNA complexes at N/P ratios in the absence (w/o) or presence (w/) of 10 mM dithiothreitol (DTT); b) Average particle sizes and zeta potentials of the complexes at an optimal N/P ratio of 20/1 in HEPES buffer (20 mM, pH 7.4).

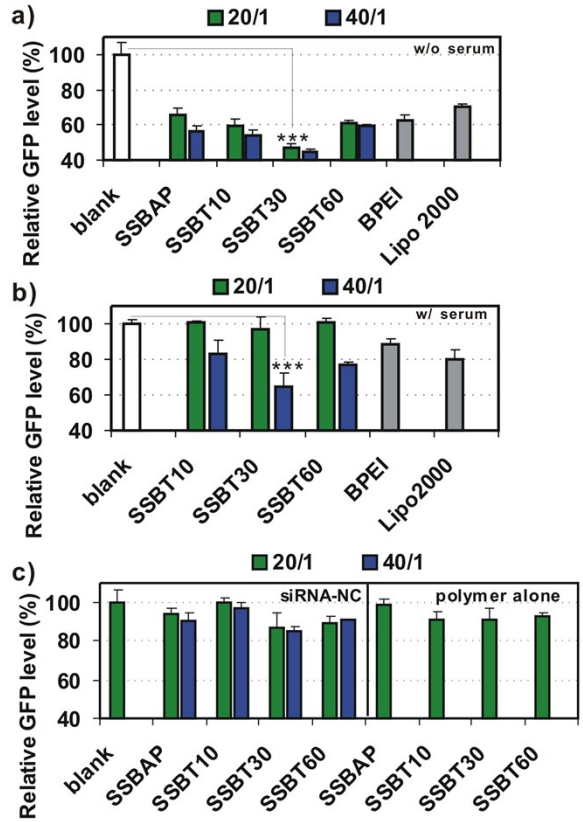

Figure S11. SSBAP and SSBT polymers for siRNA delivery into GFP-stably expressing 293T cells. a) Relative GFP expression level in GFP-stably expressing 293T cells after siRNA transfection for GFP silencing by SSBAP or SSBT copolymers at different N/P ratios using a) a serum-free or b) serum-containing transfection protocol. Lipofectamine 2000 (Lipo 2000) was used as a control; c) Relative GFP expression level in GFP-stably expressing 293T cells after the cells were treated by scrambled siRNA (siRNA-NC) transfection with SSBAP or SSBT copolymers at N/P ratios or treated only by the polymer alone with the amount as same as the case of the complexes at an N/P ratio of 20/1. *** $p<0.001$