Supplementary Information:

Reagents for Polymer Synthesis:

1-propylamine, 2-pyridinecarbaldehyde, N,N-(diethyl amino)ethyl methacrylate (DEAEM), 2-bromoisoburyryl bromide, copper(I) bromide (CuBr 99.99%), Pluronic F127 (MnZ12600, 70% PEO) and copper powder (99) for organic synthesis were purchased from Sigma-Aldrich. Triethylamine, tetrahydrofuran (THF) and toluene were purchased from Fisher Scientific and all chemicals were used with no further purification. N-Propyl-pyridynyl methanimine (NPPM) was prepared by reacting 1-propylamine with 2-pyridinecarbaldehyde. Alexafour488 was purchased from Invitrogen to prepare dye attached polymers.

Synthesis and Characterization of Pluronic Macroinitiator and Pentablock Copolymers:

The difunctional 2-bromo propionate Pluronic F127 (pluronic macroinitiator) and pentablock copolymers used in this study, PDEAEM-PEO-PPO-PEO-PDEAEM, were synthesized by atom transfer radical polymerization (ATRP), as explained in detail elsewhere [46]. The pentablock copolymers possessing different molecular weights were prepared by manipulating the DEAEM monomer amount. All ^{1}H NMR spectra were collected using a Varian VXR400 (400 MHz) spectrometer, and chemical shifts are given in ppm. Chloroform-d (98%, Fisher) was used as solvent. The copolymer molecular weight and polydispersity index (PDI) were determined using gel permeation chromatography (GPC). The GPC system consisted of a Waters 510 pump, Waters 717

autosampler, a Wyatt Optilab DSP refractometer, and a Wyatt Dawn EOS light scattering detector. The mobile phase was THF at a flow rate of 1 ml/min in the column.

The conversion of hydroxyl end groups to bromides was confirmed by ^{1}H NMR spectra (Figure S1 Supplementary information). The peak at 1.9 ppm in the spectra given for the macroinitiator corresponds to the methyl groups in the alpha position relative to the bromide. In order to calculate conversion of initiating end groups, this specific peak was integrated relative to the peaks arising from the F127 backbone protons. The reaction of amine methacrylate blocks with the macroinitiator results in the formation of pentablock copolymers. This was proved by the presence of the bonds A, E, F, G, I, J in the ^{1}H NMR spectra shown in Figures S2 and S3 as reference to the ^{1}H NMR of Pluronic F127 macroinitiator (Figure S1). The formation of pentablock copolymer was also demonstrated with gel permeation chromatography through the faster elution time as compared to the macroinitiator (Figure S4 Supplementary Information). The number and weight average molecular weight of the polymers synthesized are summarized in Table 1 in main text. The average polymer molecular weight can be controlled in a relatively low polydispersity range by manipulating the macroinitiator to monomer ratio. By keeping the macroinitiator amount constant and increasing the monomer amount, the increase in molecular weight was achieved.

Synthesis of Dye Attached Polymers:

Pentablock copolymer was amine functionalized by transforming the bromine group into azide. 3 g of pentablock copolymer was reacted with 300 mg of sodium azide in 15 mL (dimethylformamide) DMF. The reaction was carried out at 50 ºC under continuous stirring (300 rpm) during 24 h and the excess DMF was removed by rotary evaporation. The remaining polymer was dissolved in water, the excess sodium azide was removed by dialysis and the modified polymer was freeze-dried. The AlexaFluor 488 alkyne dye was attached to the azide modified polymer via copper-catalyzed azide alkyne click reaction. The azide-modified polymer (20 mg/ml) was reacted with 100 µg dye in DMF with 4.7 mg ascorbic acid and 3.325 mg copper II sulphate under continuous stirring during 2 h in the dark. The excess dye was removed by dialysis during 24 h and the purified polymer was freeze-dried.

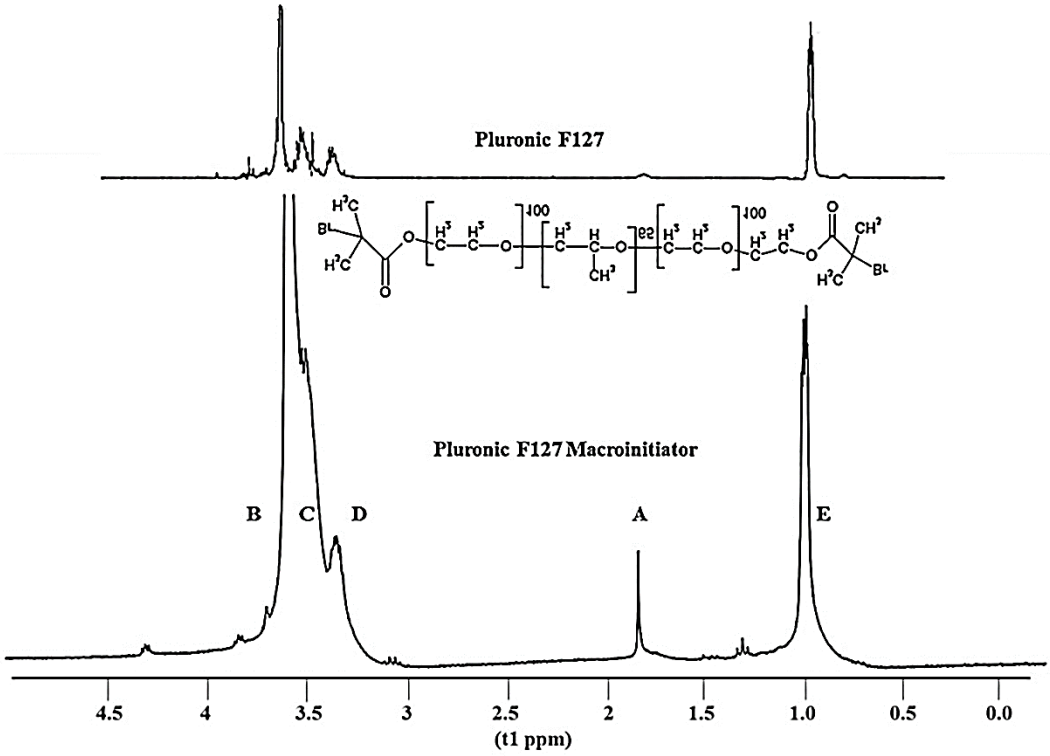

Figure S1. ^{1}H NMR of Pluronic F127 and Pluronic F127 ATRP macroinitiator.

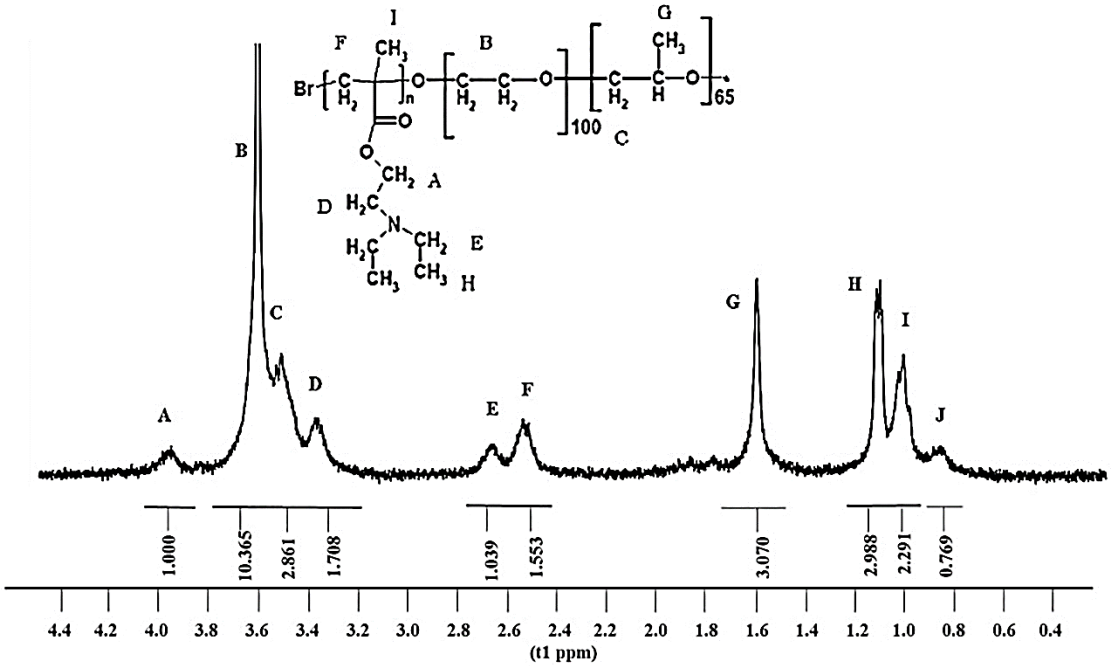

Figure S2. [1]H NMR of pentablock copolymers PDEAEM-PluronicF127-PDEAEM, Polymer A.

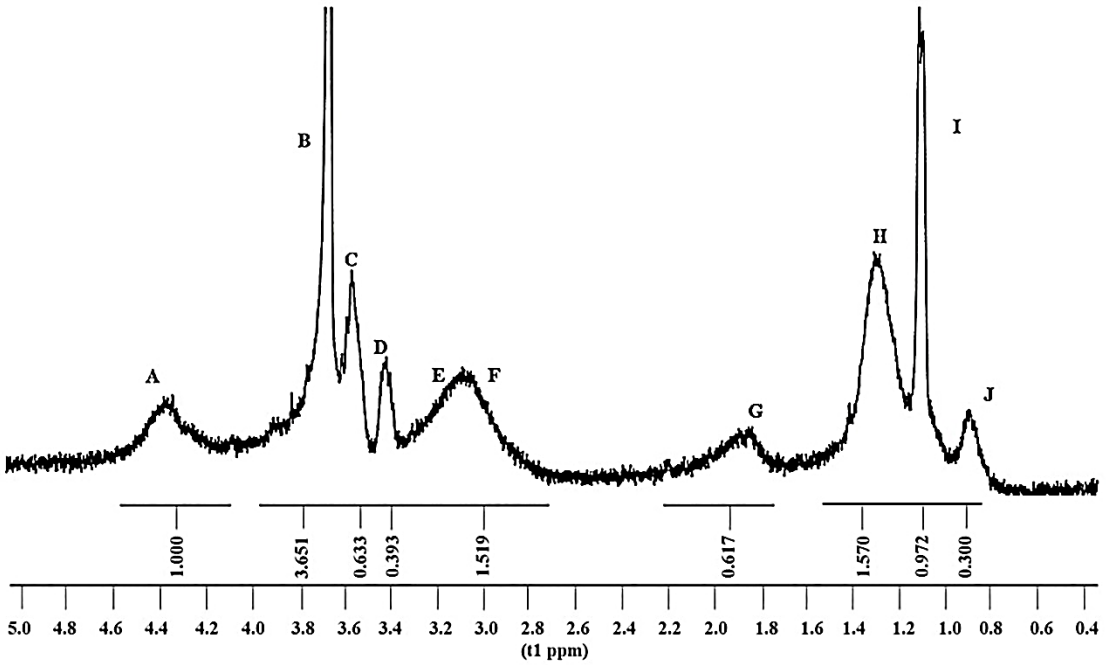

Figure S3. [1]H NMR of pentablock copolymers PDEAEM-PluronicF127-PDEAEM, Polymer C.

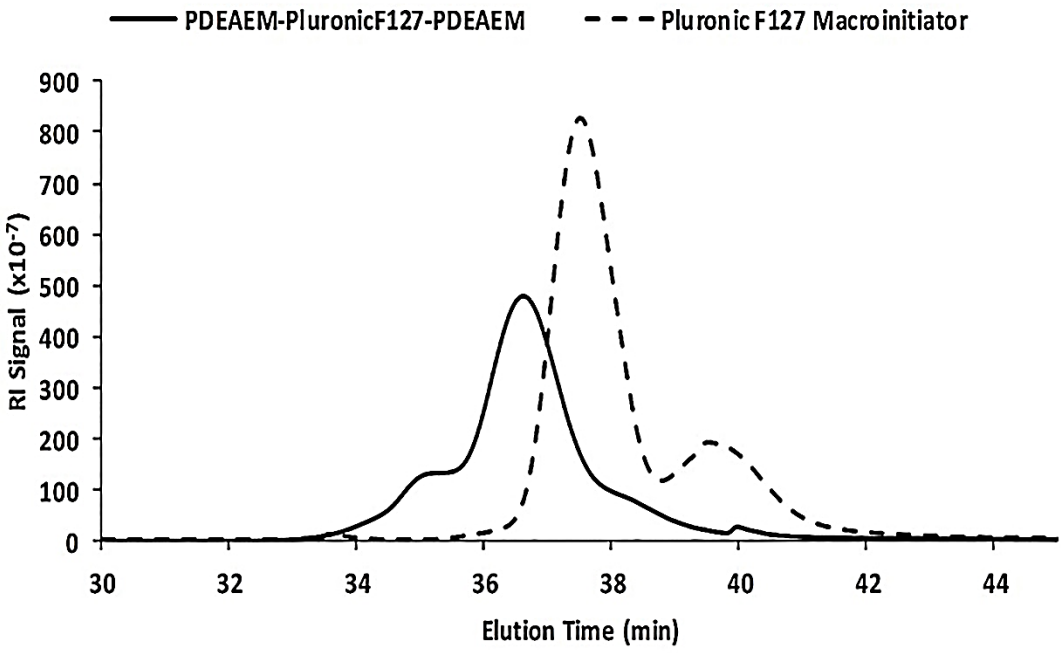

Figure S4. GPC chromatograph of Pluronic F127 macroinitiator and a pentablock copolymer.

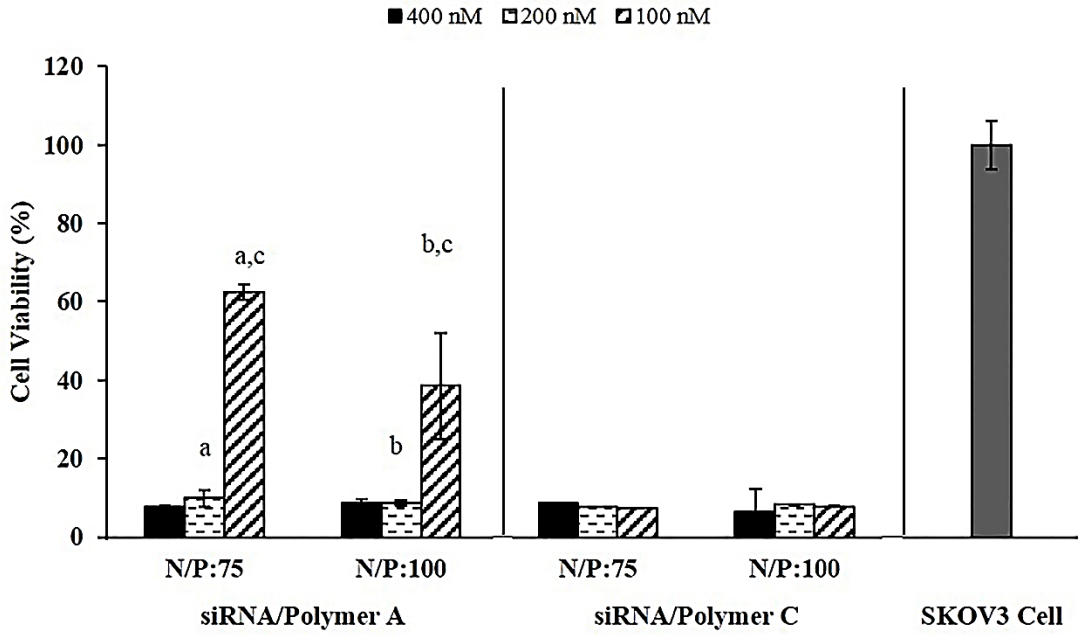

Figure S5. Cell viability of siRNA/Polymer A and siRNA/Polymer C polyplexes administered SKOV3 cells. N/P: 75 and 100. Applied siRNA dose: 100, 200, 400 nM. Initial SKOV3 cell density: $2x10^4$ cell/well. Incubation time: 24h. a, b: represent doses of siRNA/Polymer A complexes which caused significant difference in cell viability ($p<0.05$) for N/P ratios of 75 and 100, respectively. c: represents the siRNA/Polymer A complexes prepared at different N/P ratios which showed significant difference ($p<0.05$) in cell viability at dose of 100 nM.

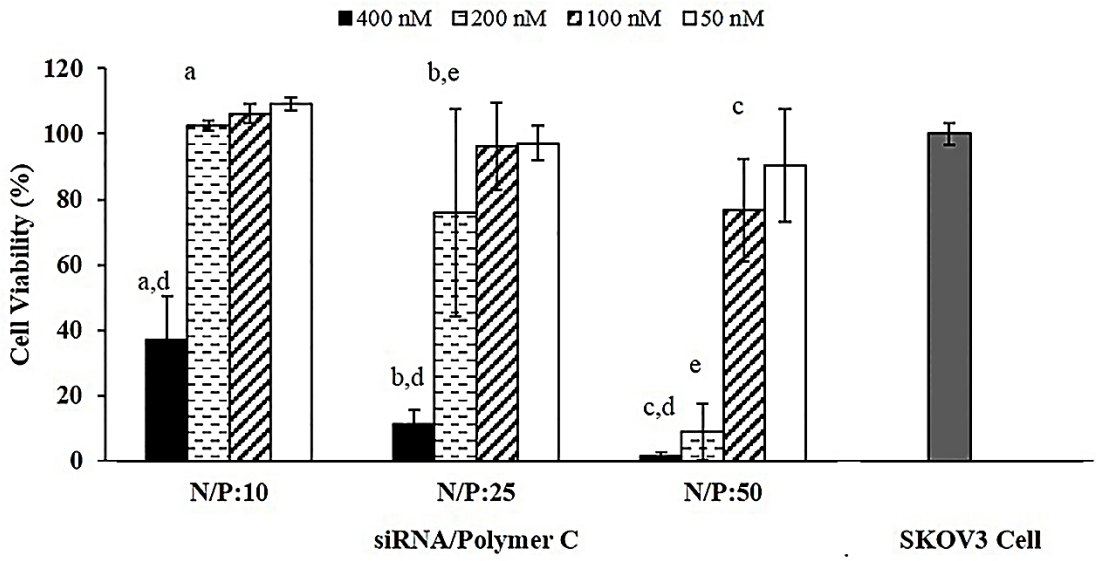

Figure S6. Cell viability of siRNA/Polymer C polyplex administered SKOV3 cells. N/P: 10, 25, 50. Applied siRNA dose: 50, 100, 200, 400 nM. Initial SKOV3 cell density: $2x10^4$ cell/well. Incubation time: 24h. a,b,c: represent doses of siRNA/Polymer C polyplexes which caused significant difference in cell viability ($p<0.05$) for all N/P ratios. d, e : represent the siRNA/Polymer C polyplexes prepared at different N/P ratios which showed significant difference ($p<0.05$) in cell viability at doses of 400 and 200 nM, respectively.

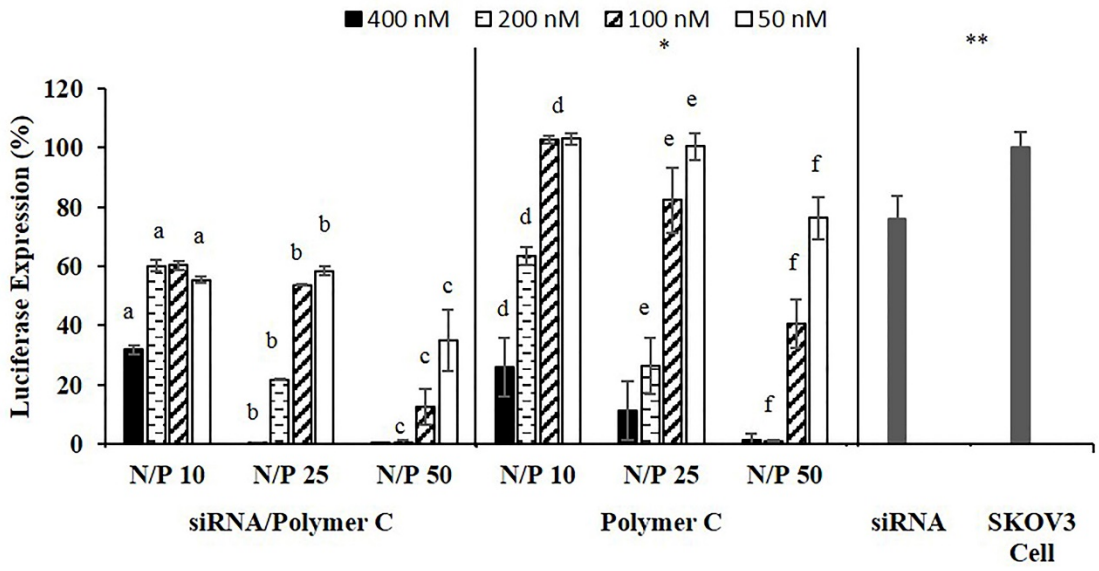

Figure S7. Luciferase suppression of prepared siRNA/Polymer C polyplexes. N/P: 10, 25, 50. Applied siRNA dose: 50, 100, 200, 400 nM. Initial SKOV3 cell density: $2x10^4$ cell/well. Controls: Amount of Polymer C required to form polyplexes possessing N/P ratio 10, 25, 50 (without siRNA), naked siRNA, SKOV3 control cell. Incubation time: 24h. For each N/P ratio, the same letters on the bars represent doses of siRNA/Polymer C polyplexes or Polymer C alone which caused significant difference in luciferase expression suppression ($p<0.05$). *Compared to siRNA/Polymer C polyplexes, Polymer C alone showed significantly lower luciferase expression suppression ($p<0.05$) at 50 and 100 nM doses for all N/P ratios. ** The luciferase expression suppression values obtained with 400 nM naked siRNA or SKOV3 cells were also found significantly lower ($p<0.05$) than the suppression provided by 400 nM siRNA/Polymer C polyplex prepared at N/P ratio of 50.

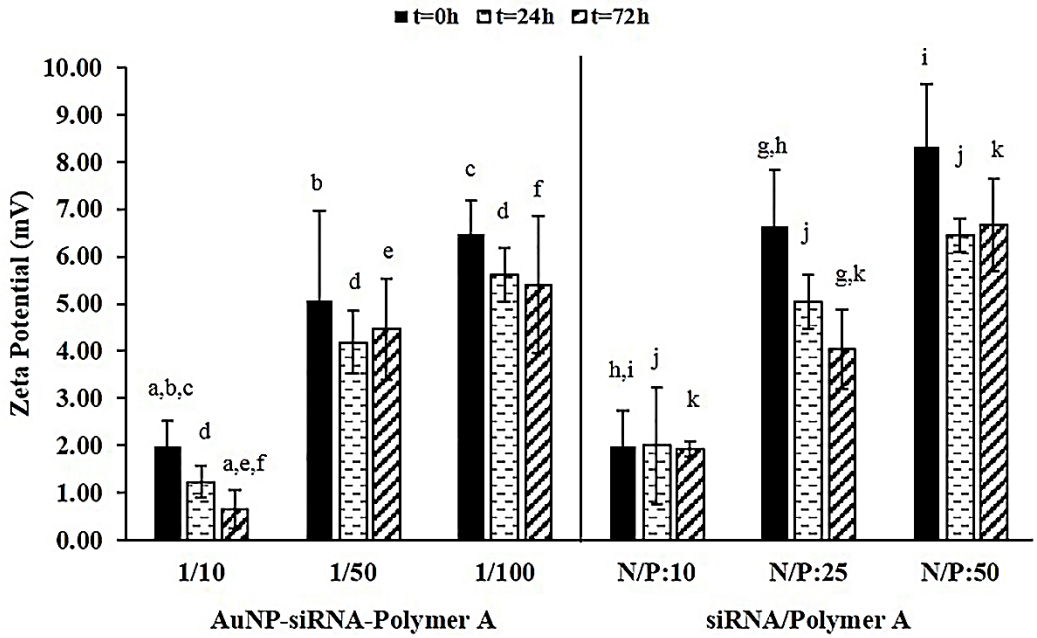

Figure S8. Change in zeta potential of AuNP-siRNA-Polymer A conjugates with AuNP/Polymer ratios of 1/10, 1/50, 1/100 and siRNA/Polymer A complexes with N/P ratios of 10, 25 and 50 with respect to time in serum containing cell culture media. Same letters on the bars represent the significant difference ($p<0.05$).

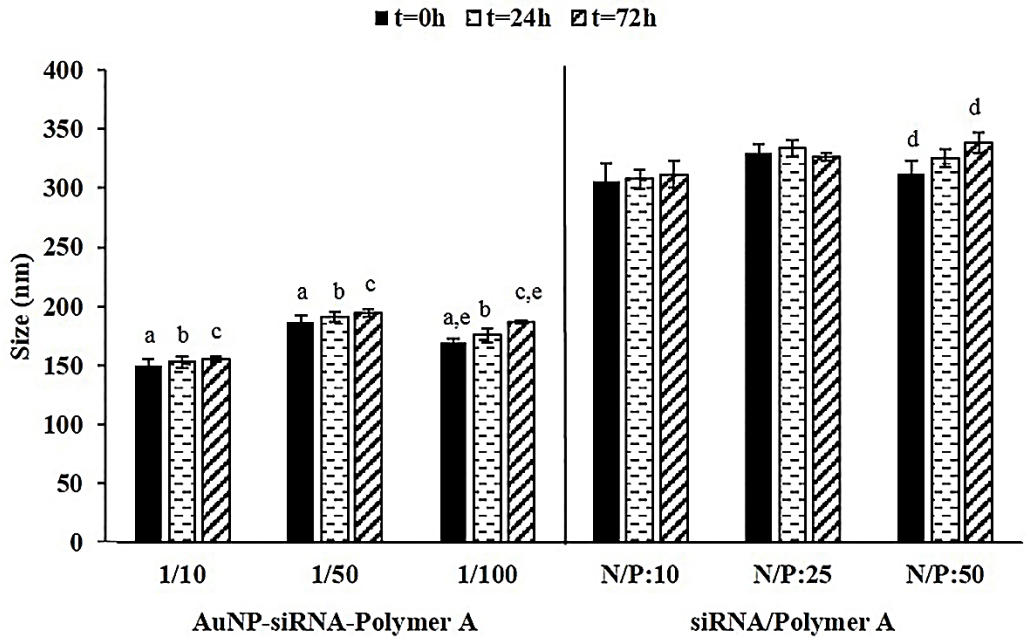

Figure S9. Change in size of AuNP-siRNA-Polymer A conjugates with AuNP/Polymer ratios of 1/10, 1/50, 1/100 and siRNA/Polymer A complexes with N/P ratios of 10, 25 and 50 with respect to time in serum containing cell culture media. Different letters on the bars represent significant difference ($p<0.05$).

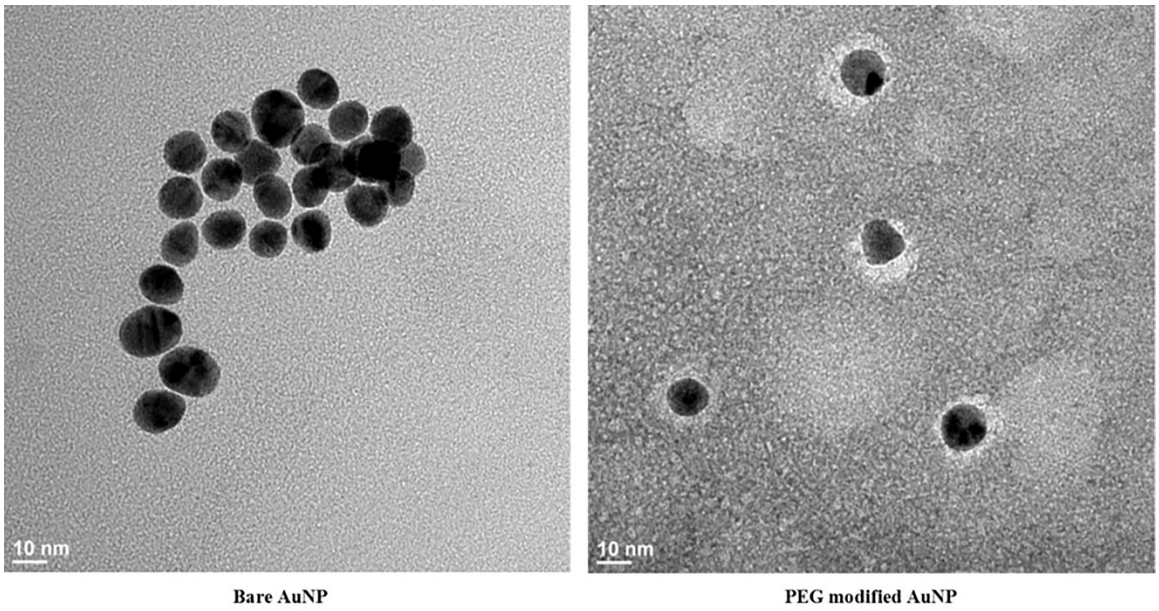

Figure S10. TEM images of bare and PEG modified AuNPs.

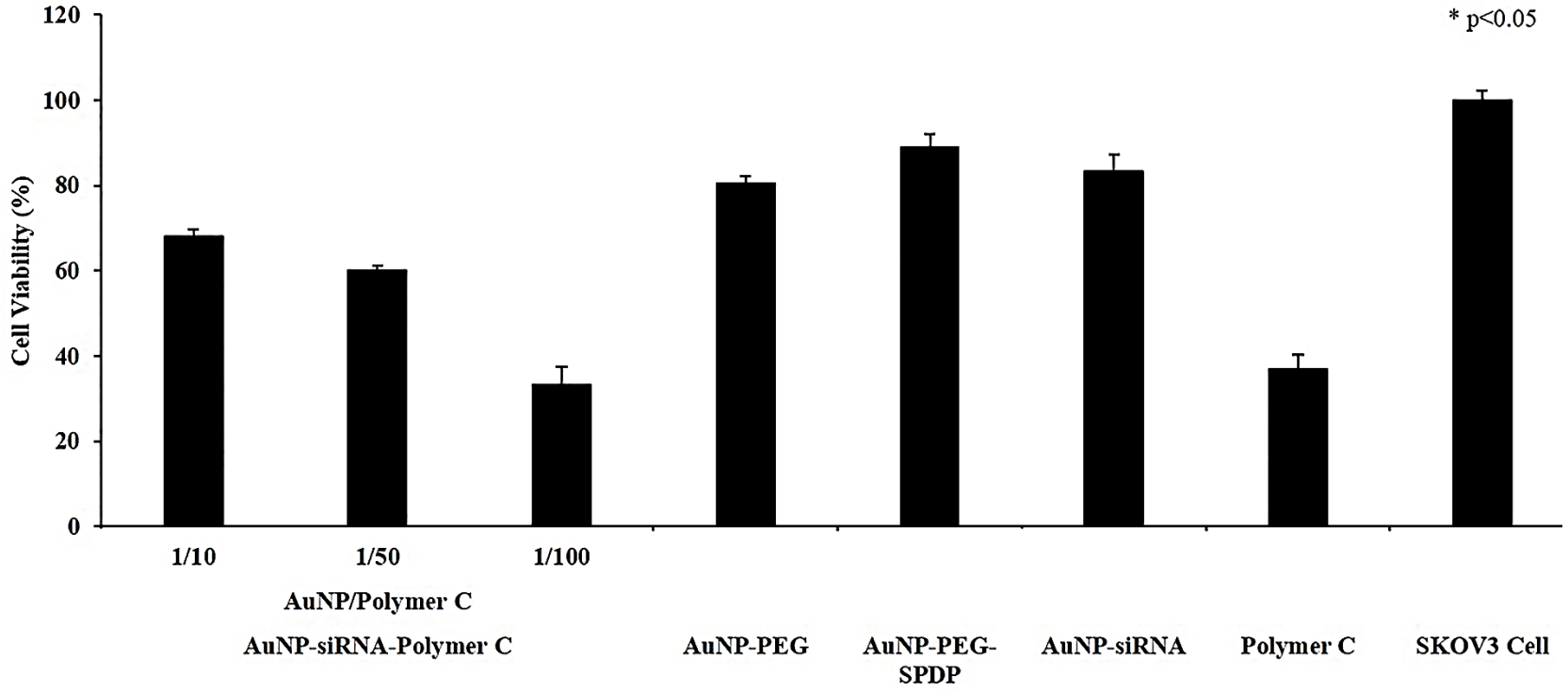

Figure S11. Cell viability of AuNP-siRNA-Polymer C MCS treated SKOV3 cells. Applied MCS dose (based on AuNP amount): 5 nM/well. Applied siRNA dose: 100 nM/well. Applied polymer dose: 500 nM/well. Initial SKOV3 cell density: $1.5x10^4$ cell/well. Controls: AuNP-PEG, AuNP-PEG-SPDP, uncoated AuNP-siRNA (without Polymer C), Polymer C alone, control SKOV3 cell. Incubation time: 24h. * AuNP-siRNA-Polymer C MCS prepared with different polymer amounts, the layers of this multicomponent system (AuNP-PEG, AuNP-PEG-SPDP and AuNP-siRNA) and Polymer C alone caused significantly different cell viabilities compared with the SKOV3 cell alone ($p<0.05$).

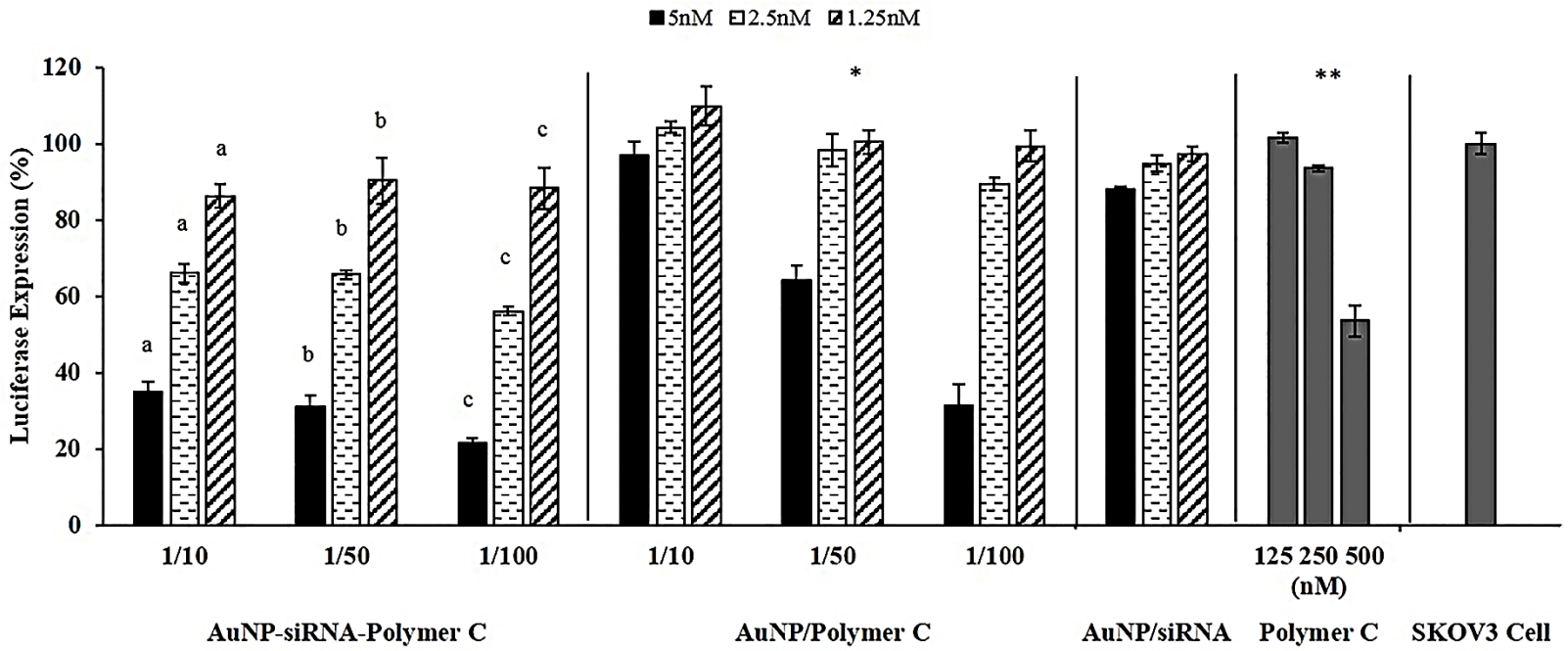

Figure S12. Luciferase suppression of prepared AuNP-siRNA-Polymer C MCS. Applied MCS dose (based on AuNP amount): 1.25, 2.5, 5 nM/well. Applied siRNA dose: 25, 50, 100 nM/well. Initial SKOV3 cell density: $1.5x10^4$ cell/well. Controls: AuNP-Polymer C (without siRNA), uncoated AuNP-siRNA MCS (without Polymer C), Polymer C alone, SKOV3 cell. Incubation time: 24h. a,b,c: Represent doses of AuNP-siRNA-Polymer C MCS which caused significant difference in luciferase expression ($p<0.05$) for AuNP/Polymer ratios of 1/10, 1/50 and 1/100, respectively. *: Compared to AuNP-siRNA-Polymer C MCS, AuNP/Polymer C MCS showed significantly lower luciferase expression suppression ($p<0.05$) at all AuNP/Polymer ratios and doses applied. **: Polymer C

alone applied at 125 and 250 nM doses showed significantly lower luciferase expression suppression than AuNP-siRNA-Polymer C MCS prepared with AuNP/Polymer ratio of 1/100.

Theoretical siRNA Loading on AuNPs:

AuNP Concentration: 20 nM
siRNA Molecular Weight: 13600 g/mole
Reaction volume: 500 µl
AuNP diameter: ~13 nm
siRNA diameter: ~2 nm
siRNA length: ~7.5 nm
N_A: $6.02x10^{23}$

$$\frac{20nmole}{10^6\,\mu l}\,x\,500\mu l\,x\,\frac{6.02\,x\,10^{23}\#of\;AuNP}{1\;mole}\,x\,\frac{1\;mole}{10^9\;nmole}=6x10^{12}\;\#\;of\;AuNP$$

$$Surface\;Area\;of\;1\;AuNP=4\;x\;3.14\;x\;(6.5)^2=530\;nm^2$$

$$Total\;Surface\;Area\;of\;AuNPs=530\frac{nm^2}{1\;AuNP}\,x\,6x10^{12}\#\;ofAuNP=318x10^{13}\;nm^2$$

$$Area\;of\;1\;siRNA\;Molecule=4\;x\;3.14\;x\;(1)^2=12\;nm^2$$

$$\frac{530\;nm^2}{12\;nm^2}=44\;\#\;of\;siRNA\;required\;to\;fully\;coat\;the\;surface\;of\;1\;AuNP$$

$$\frac{318x10^{13}\;nm^2}{12\;nm^2}=26x10^{13}\;total\;\#\;of\;siRNA$$

$$26x10^{13}\;\#\;of\;siRNA\;x\;\frac{1\;mole}{6.02\;x\;10^{23}\#of\;siRNA}=4.4\;x\;10^{-10}\;mole\;siRNA\;in\;total$$

$$\frac{4.4\;x\;10^{-10}\;mole}{500\;\mu l}\,x\,\frac{10^6\mu l}{1\;L}=0.88x10^{-6}\;M=880\;nM$$

Experimental siRNA Loading on 20nM AuNP Solution:

Measured average siRNA fluorescence value : 851
Dilution factor: 20
Slope of the calibration curve: 2236

$$siRNA\ Concentration = \frac{851}{2236} = 0.38\ x\ 20\ (dilution\ factor) = 7.62\ \frac{\mu g}{ml}$$

$$\frac{7.62\ \mu g}{1000\ \mu l}\ x\ \frac{1\ mole}{13600\ g}\ x\ \frac{1g}{10^6 \mu g}\ x\ \frac{10^6 \mu l}{1L} = 0.056x10^{-5}\ M = 560\ nM$$

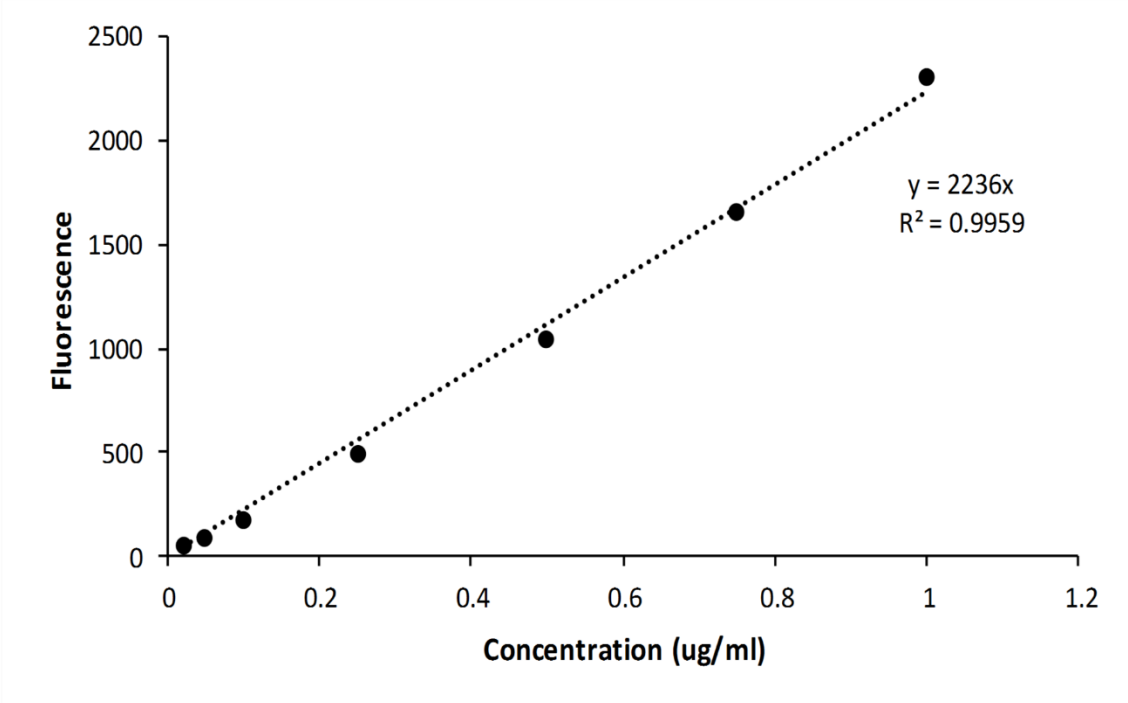

Figure S13. Calibration curve for the determination of siRNA quantity.

Calculation of Number of AuNPs per Cell by ICP-MS Analysis:

Measured Au Concentration by ICP-MS : 0.323 mg/L

Number of Au atoms in measured sample:

$$Number\ of\ Au\ atoms = 0.323\frac{mg}{L}x\frac{1\ mol}{197\ g}x\frac{1\ g}{1000\ mg}x\frac{6.02x10^{23}}{1\ mol}x\frac{1\ L}{1000\ ml}x20ml$$

$$Number\ of\ Au\ atoms = 1.97x10^{17}$$

Number of Au atoms to form one AuNP:

$$N = \left(\frac{Radius\ of\ AuNP}{Radius\ of\ Au\ atom}\right)^3 = \left(\frac{6.5}{0.137}\right)^3 = 106801\ Number\ of\ Au\ atoms$$

Number of AuNPs in measured sample:

$$Number\ of\ AuNPs = \frac{1.97x10^{17}}{106801} = 1.85x10^{12}$$

Number of AuNPs in cell:

Number of cells : $1x10^5$ cell

$$Number\ of\ AuNP\ per\ cell = \frac{1.85x10^{12}}{1x10^5} = 1.85x10^7$$

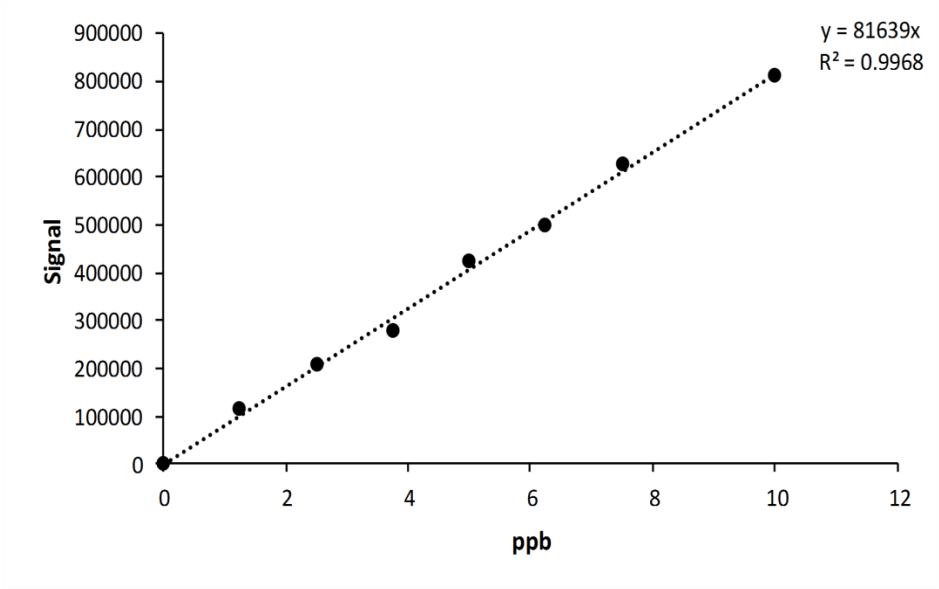

Figure S14. Calibration curve for the determination of Au atoms by ICP-MS.