(Supporting Information)

Scavenger Receptors Mediate Cellular Uptake of Polyvalent Oligonucleotide-Functionalized Gold Nanoparticles

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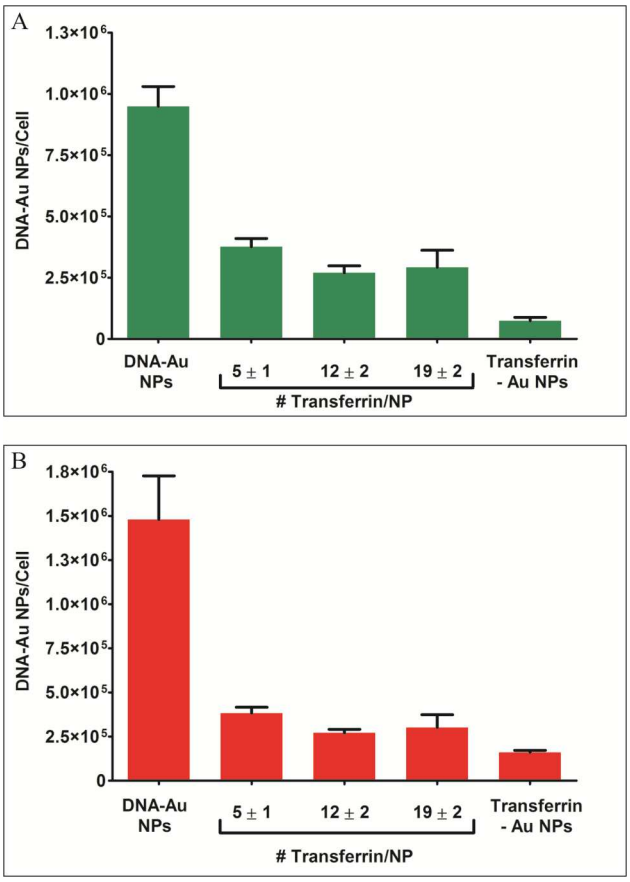
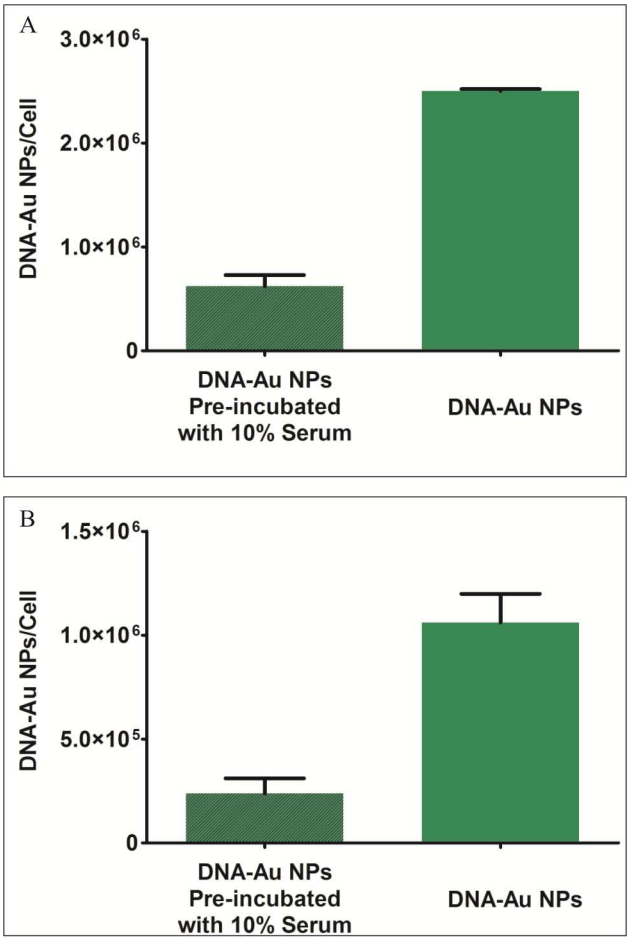
EXPERIMENTAL PROCEDURES

Nanoparticle stability in poly I and fucoidan solutions. DNA and siRNA functionalized gold nanoparticles were prepared as described in experimental procedures. Poly I and fucoidan solutions were prepared in phosphate buffered saline. DNA-Au NPs and siRNA-Au NPs were incubated with poly I and fucoidan at solution concentration of 10nM for 24 hours at 37°C. Nanoparticles without any additional chemicals were used as controls. Following the incubation, the stability of nanoparticles was measured using Cary 5000 UV-VIS-NIR instrument, scanning between 400-700nm wavelengths. The resultant spectra were normalized to the gold SPR peak.

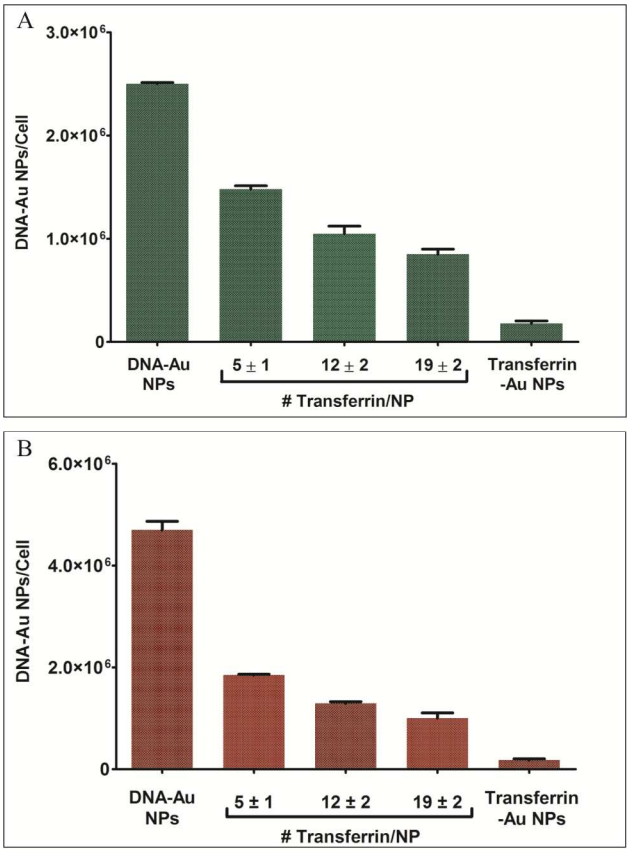
Quantitative real-time polymerase chain reaction. HeLa cells were plated in 48-well plate at density of 25000 cells/well. Following overnight incubation, the cells were treated with 25nM and 50nM of siRNAs targeting scavenger receptors A and B1 (Santa Cruz) using lipofectamine transfection reagent. After 24 hours, the siRNA containing medium was replaced with regular growth medium, EMEM with 10% FBS. The cells were allowed to grow for another 24 hours after which the experiment was terminated. Total RNA was extracted using TRIzol (Invitrogen) following manufacturer's recommended protocol. One μg of this RNA was used for cDNA synthesis using Taqman Gold RT-PCR kit (Applied Biosystems). Real-time PCR was performed on a Roche 480 Lightcycler. Hydrolysis probes (Roche) were used to measure the relative abundance of each target mRNA. These values were normalized to actin expression and compared to cells treated with lipofectamine alone to determine the change in mRNA levels. The primers used in these experiments were, SR-A left, 5' -TTT GAT GCT CGC TCA ATG AC- 3', SR-A right, 5' -TGA AGG GAA GGG CTG TTT TT- 3', SR-B1 left, 5' -CAT CAA GCA GCA GGT CCT TA- 3', SR-B1 right, 5' – CGG AGA GAT AGA AGG GGA TAG G- 3'. Actin primers are probes were purchased as single reagent from Roche.



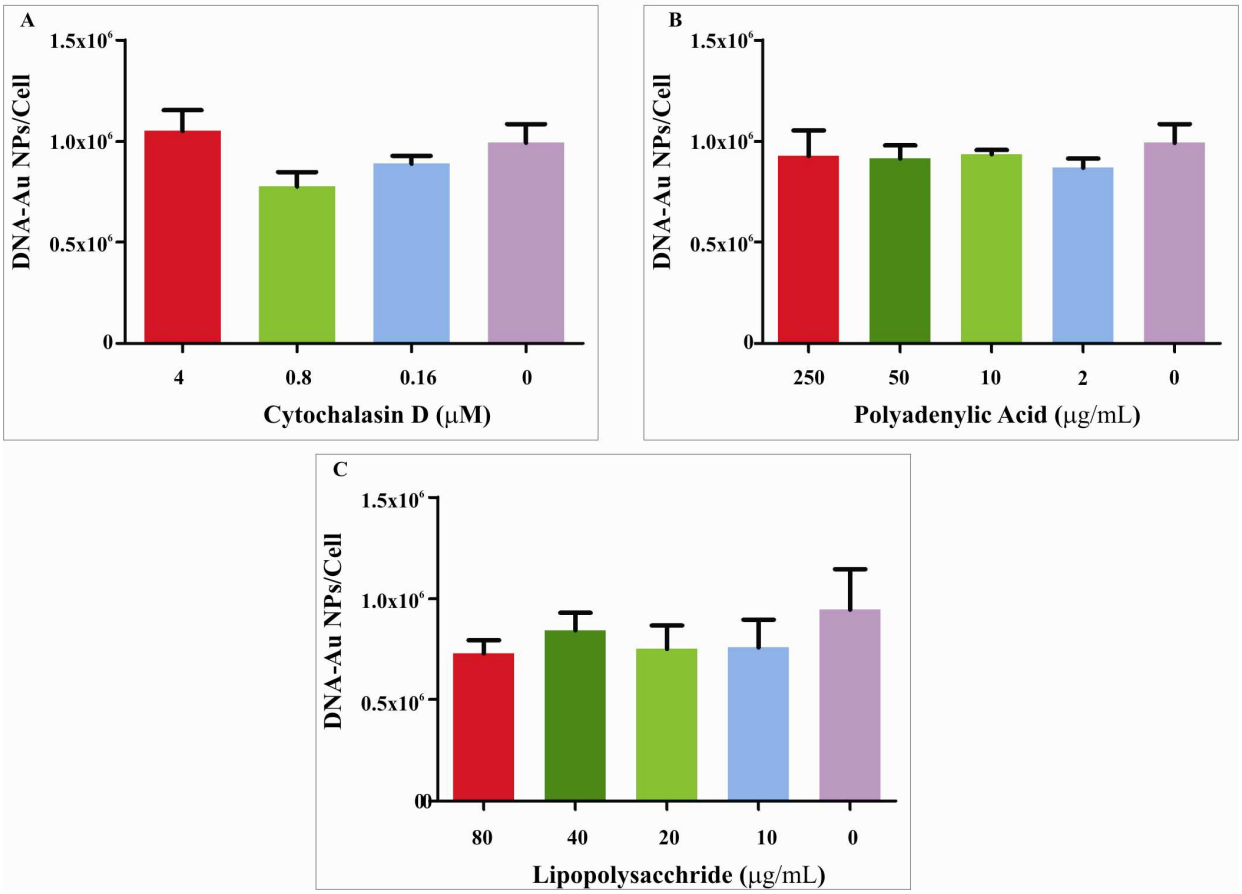
Supporting Figure 1. Cellular uptake of DNA-Au NPs coated with varying numbers of transferrin molecules. The method for loading a variable number of proteins on DNA-Au NPs is shown in Scheme 1. Cells were grown in media containing 10% FBS with 5 nM (green) or 10 nM (red) concentration of nanoparticles.



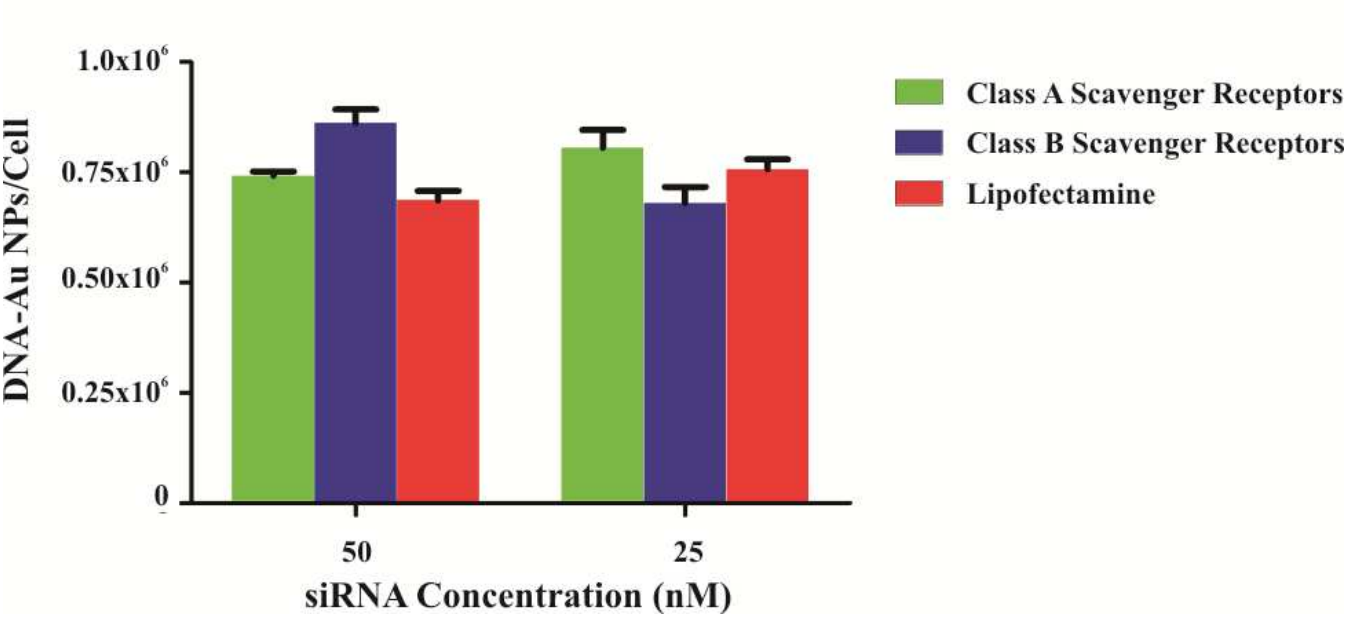
Supporting Figure 2. (A) Cellular uptake of DNA-Au NPs (5nM) with or without serum pre-incubation in normal culturing conditions (EMEM+10% FBS). Pre-incubation of DNA-Au NPs with serum reduces cellular uptake by 76% when compared direct addition without pre-incubation. (B) Comparison of cellular uptake of DNA-Au NPs in serum-free and normal 10% FBS cultures. The serum-free conditions yield 150% higher nanoparticle uptake.



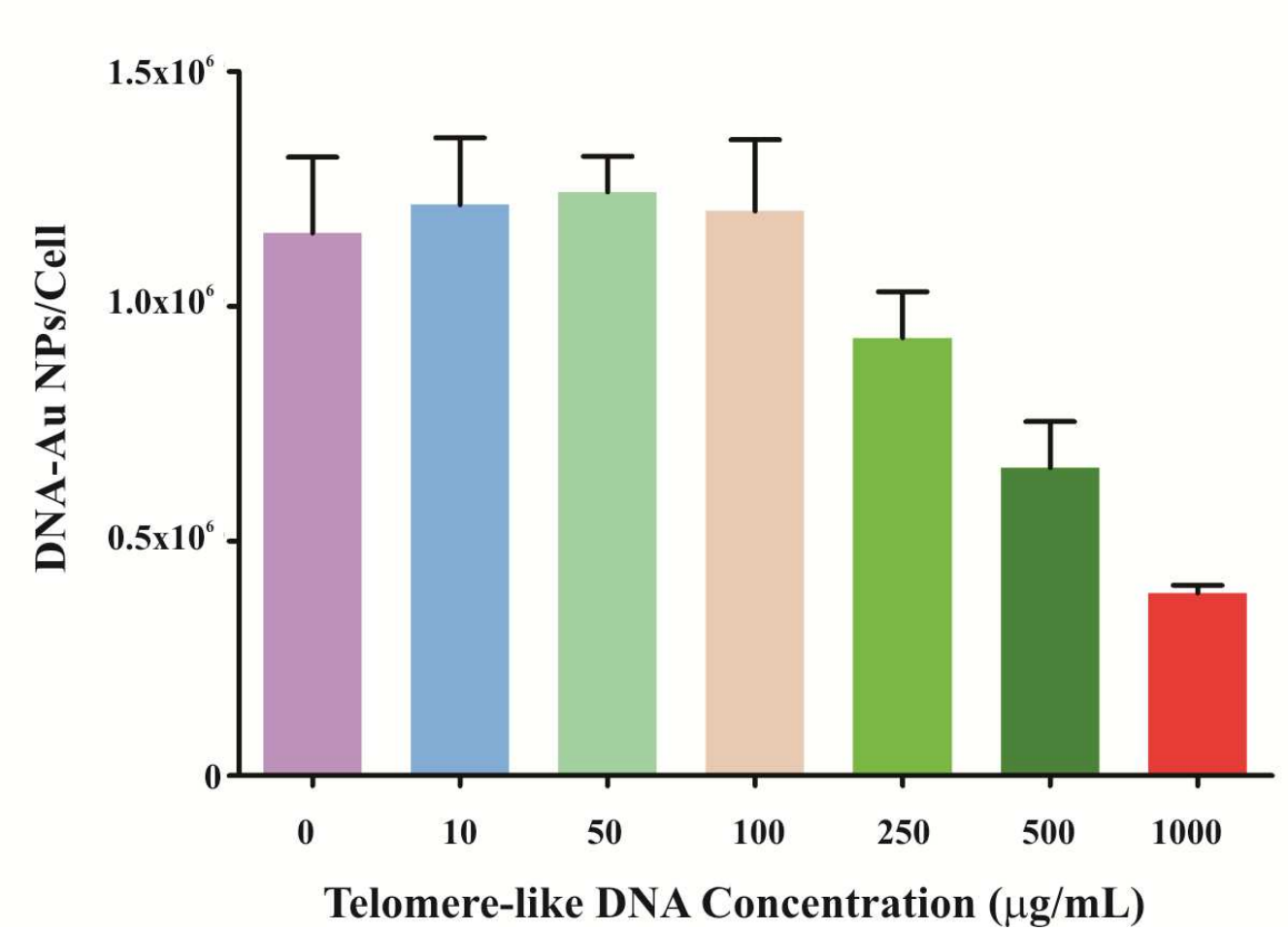
Supporting Figure 3. Cellular uptake of DNA-Au NPs when coated with varying numbers of transferrin molecules. The method for loading variable number of proteins on DNA-Au NPs is shown in Scheme 1. Cells were grown in serum-free conditions with 5 nM (green) or 10 nM (red) concentration of nanoparticles. Nanoparticle uptake is significantly higher than in normal serum culturing conditions (Supp. Figure 1).



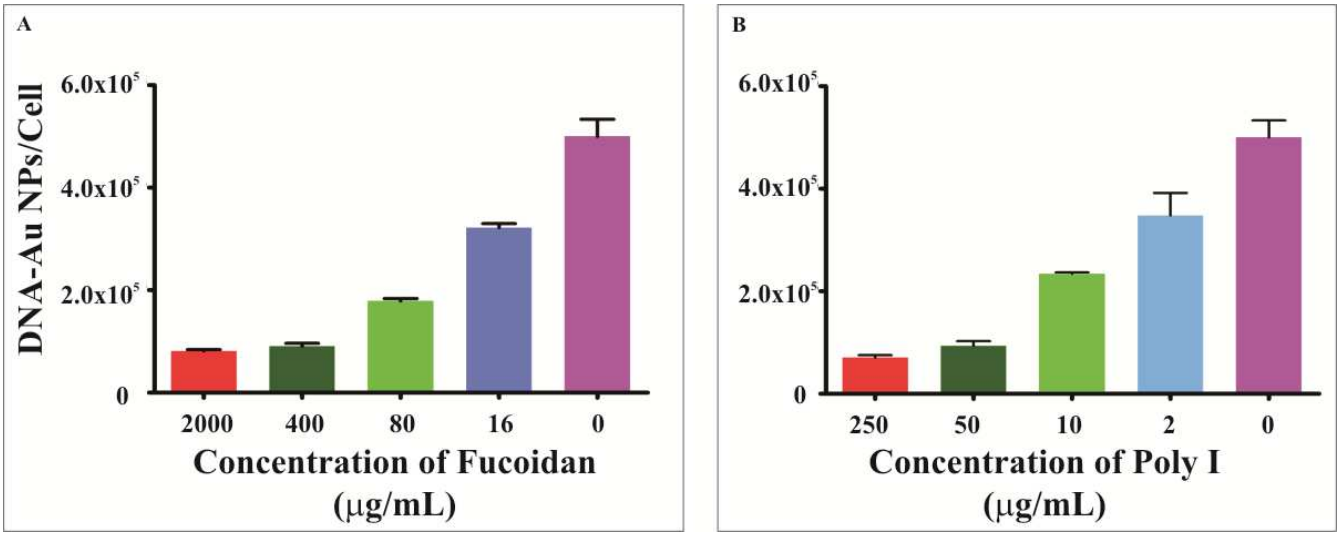
Supporting Figure 4. Cellular uptake of DNA-Au NPs in HeLa cells treated with inhibitors of endocytotic pathways. High cellular uptake in the presence of Cytochalasin D (A) suggests that DNA-Au NPs do not depend on phagocytosis as the primary mode of cellular entry. The cellular entry is inhibited in the presence of agonists of scavenger receptors, such as fucoidan and polyinosinic acid but not in the presence of chemically related molecules that do not bind scavenger receptors, such as polyadenylic acid (B) and lipopolysacchrides (C).



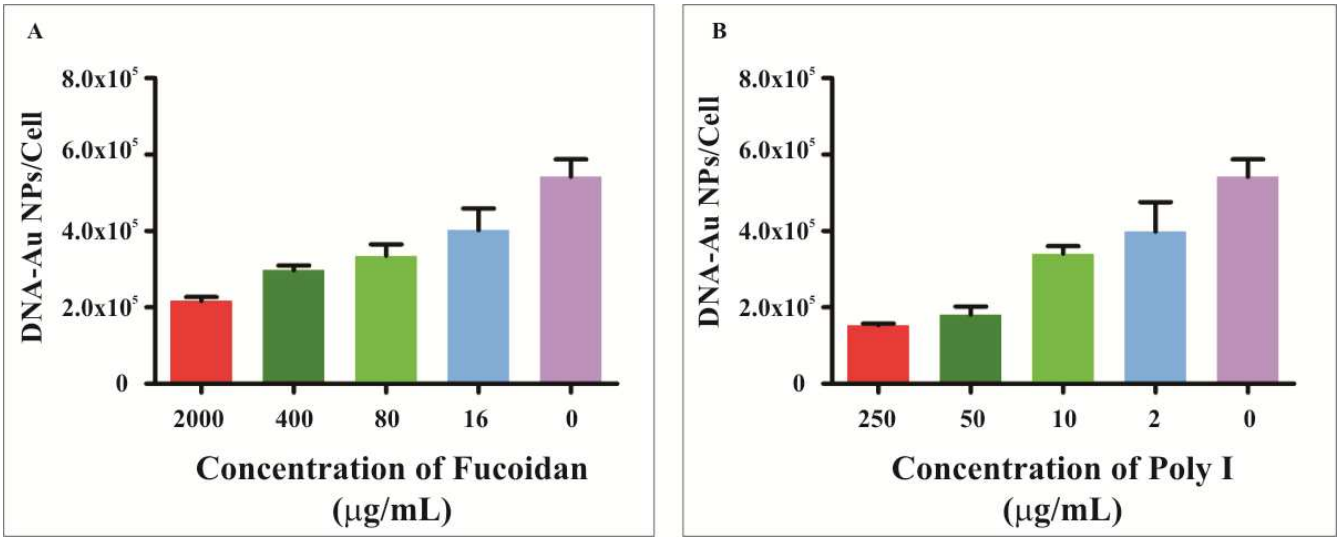
Supporting Figure 5. RNAi mediated knockdown of class A and class B scavenger receptors have no significant effect on cellular entry of DNA-Au NPs. Identical volumes of lipofectamine were used as control for each siRNA concentration. Equal volume of the two siRNA pools had no significant effect on nanoparticle uptake (data not shown).



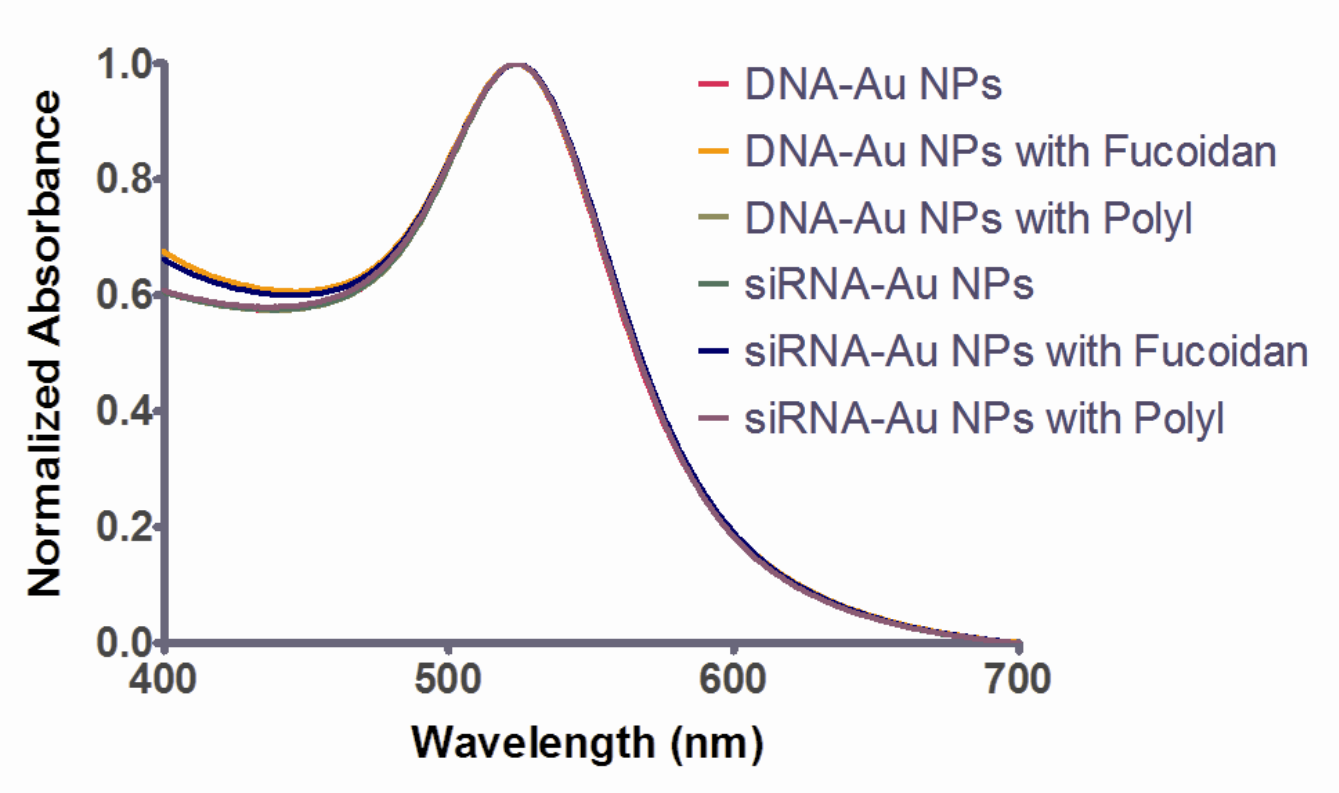
Supporting Figure 6. Synthetic ligands of scavenger receptors inhibit cellular entry of DNA-Au NPs in HeLa cells. Telomere-like DNA is theorized to form complex quadraplex-like structure that may mimic solution structure of polyinosinic acid. Higher concentration of telomere-like DNA is needed to inhibit nanoparticle entry into HeLa cells.



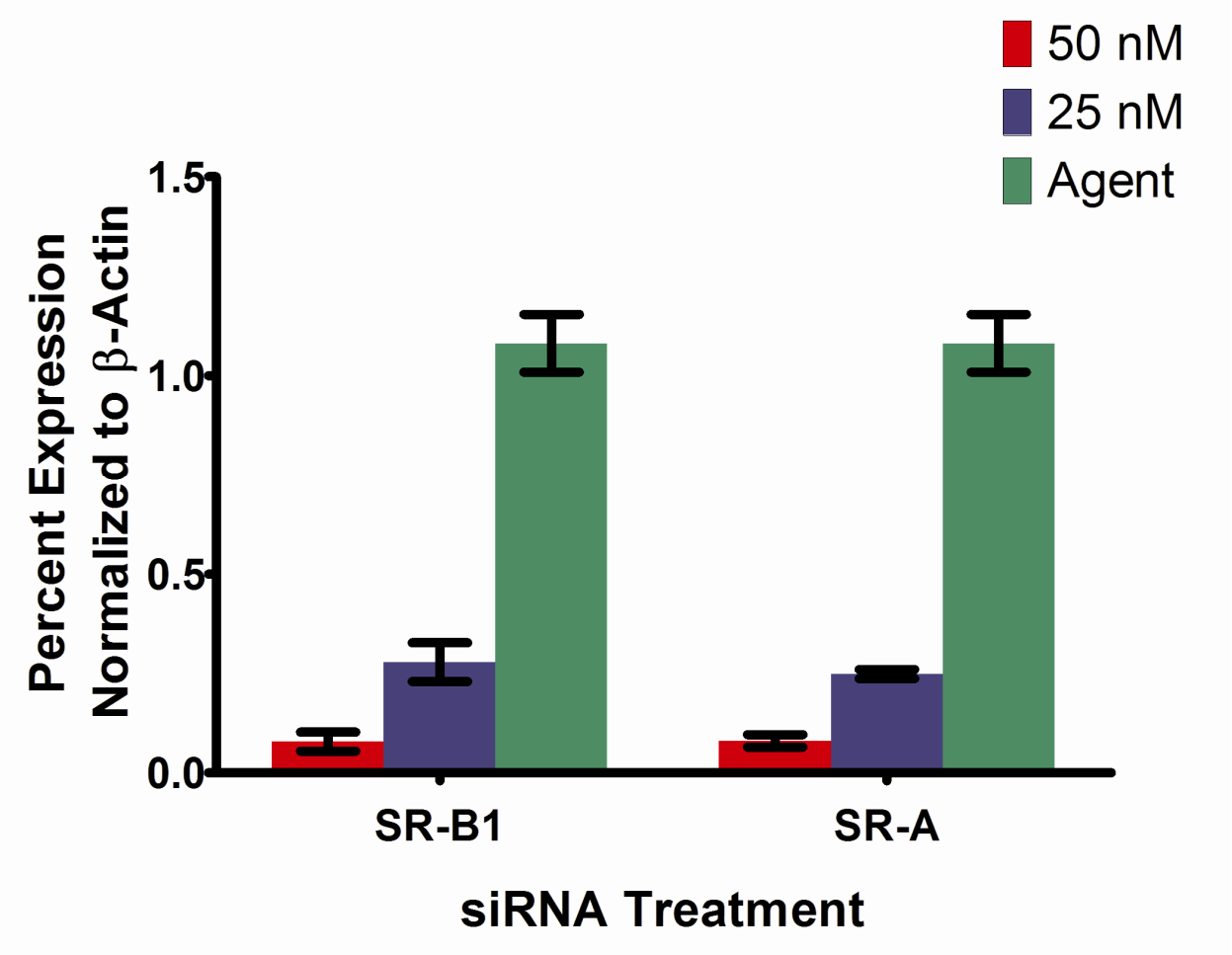
Supporting Figure 7. Cellular uptake of siRNA-functionalized nanoparticles (siRNA-Au NPs) is strongly inhibited by ligands of scavenger receptors. Both polyinosinic acid and fucoidan reduced siRNA-Au NPs entry into HeLa cells. The extent of inhibition was greater for siRNA-Au NPs than for DNA-Au NPs.



Supporting Figure 8. Cellular uptake of DNA-Au NPs in mouse cells (C166) is inhibited by ligands of scavenger receptors.



Supporting Figure 9. Stability of DNA and siRNA functionalized gold nanoparticles in the presence of Fucoidan (2000μg/mL) and Poly I (250μg/mL). The nanoparticles were incubated with the chemicals at 37℃ for 24 hours. The spectra are normalized to the SPR peak at 522nm.



Supporting Figure 10. RNAi mediated knockdown of class A and class B scavenger receptors as measured by quantitative real-time PCR. Beta actin is used as an internal control and the results are normalized to cell treated with lipofectamine treatment.

Chemical Treatment	% Cell Viability
Lipopolysacchrides (80 μg/mL)	91.7 ± 0.42
Fucoidan (2000 μg/mL)	91.9 ± 2.9
Poly I (250 μg/mL)	93.2 ± 1.3
Poly A (250 μg/mL)	93.5 ± 1.1
Untreated Cells	99.2 ± 0.85

Supporting Table 1. Cell viability as a result of treatment with highest concentration of various chemicals. Untreated cells were not given any chemicals or nanoparticles. Poly I and Poly A are similar polymers and show comparable effects on cell viability as do LPS and Fucoidan. Poly A and LPS treatments do not inhibit cellular uptake of DNA-Au NPs whereas Poly I and Fucoidan treatments inhibit internalization of DNA-Au NPs.