Supporting Information

Isothermal Self-Assembly of Spermidine/DNA Nanostructure Complex as A Functional Platform for Cancer Therapy

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DNA and RNA sequences

Strand P1 (ultraPAGE purified): 5'-GTC CTT CAA TCT CTA T CAT TCC GTC CTT CAA TCT CTA T CAT TCC GTC CTT CAA TCT CTA T CAT TCC-3';

Strand P2(PAGE purified): 5'-GAT ACC ATG AAC CAT T TAG AGA TTG AAG GAC GGA ATG T ATC AGT GTC ACT CTC GCT TGC-3';

Strand P2' (PAGE purified): 5'-GAT ACC ATG AAC CAT T TAG AGA TTG AAG GAC GGA ATG T GCA AGC GAG AGT GAC ACT GAT-3';

Strand P2-NC(PAGE purified): 5'-TTT TGT GTA GTA CAA T TAG AGA TTG AAG GAC GGA ATG T ATC AGT GTC ACT CTC GCT TGC-3';

Strand P2'-NC(PAGE purified): 5'-TTT TGT GTA GTA CAA T TAG AGA TTG AAG GAC GGA ATG T GCA AGC GAG AGT GAC ACT GAT-3';

Cy3 tagged P1(HPLC purified): 5'-Cy3-GTC CTT CAA TCT CTA T CAT TCC GTC CTT CAA TCT CTA T CAT TCC GTC CTT CAA TCT CTA T CAT TCC-3'.

mTOR siRNA sequence was 5′-AUG GUU CAU GGU AUC UUG GTT-3′

Negative control siRNA (NC) sequence was 5′-UUG UAC UAC ACA AAA GUA CUG-3′

mTOR forward primer: 5'-ACTGGAGGCTGATGGACACA-3' (Tm: 58.8 °C)

mTOR reverse primer: 5'-GGCTCTCCAAGTTCCACACC-3' (Tm: 58.9 °C)

β-actin foward primer: 5'-TCAGGTCATCACTATCGGCAAT-3' (Tm: 56.1 °C)

β-actin reverse primer: 5'-AAAGAAAGGGTGTAAAACGCA-3' (Tm: 53.9 °C).

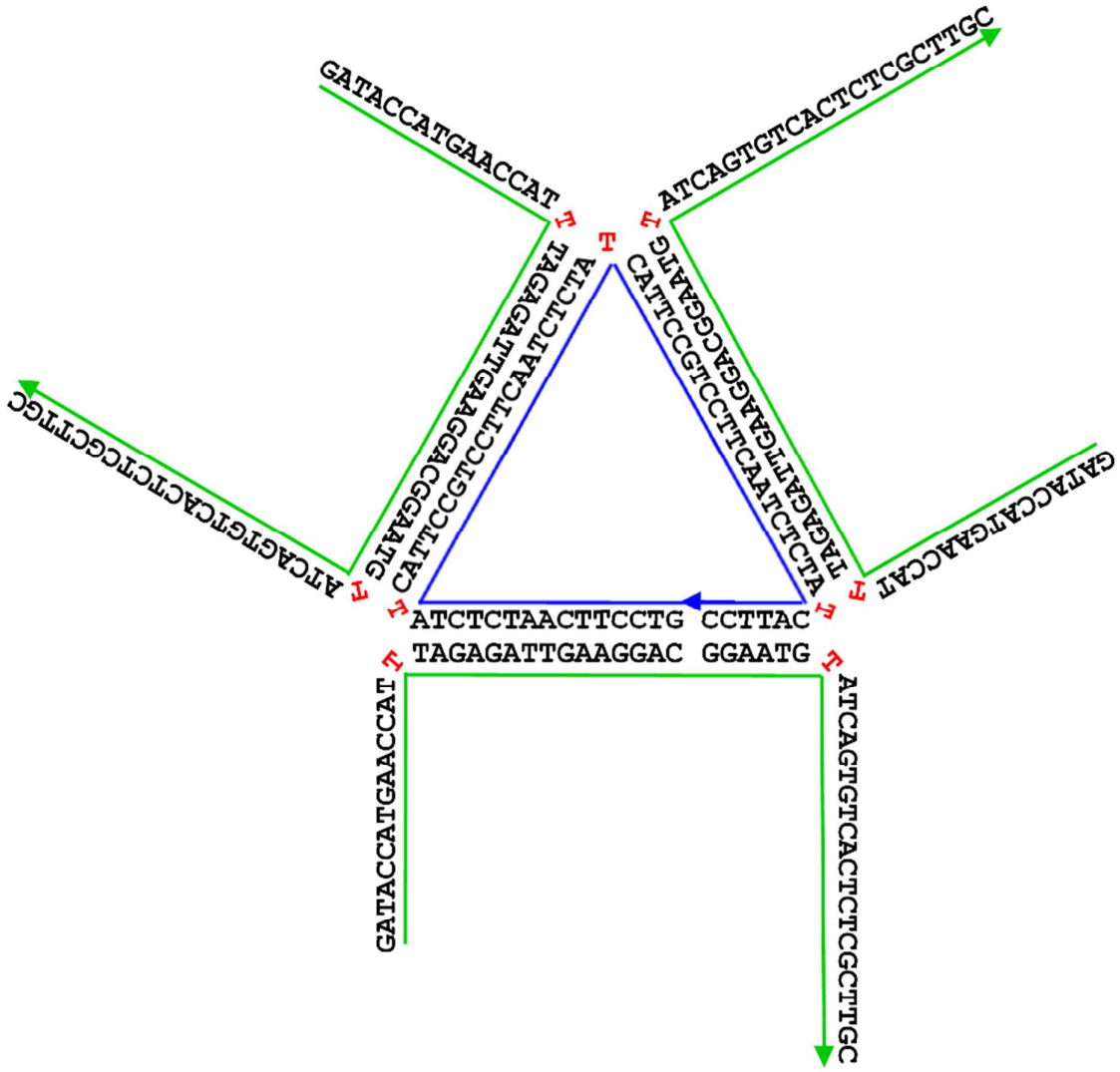

Figure S1 The sequences for the triangular-shaped structure. It consists of one central strand P1 and three copies of side strand P2. One protruding segment of P2 (at 5' end) hybridize to mTOR siRNA; the other segement is supposed to linked to another DNA triangle.

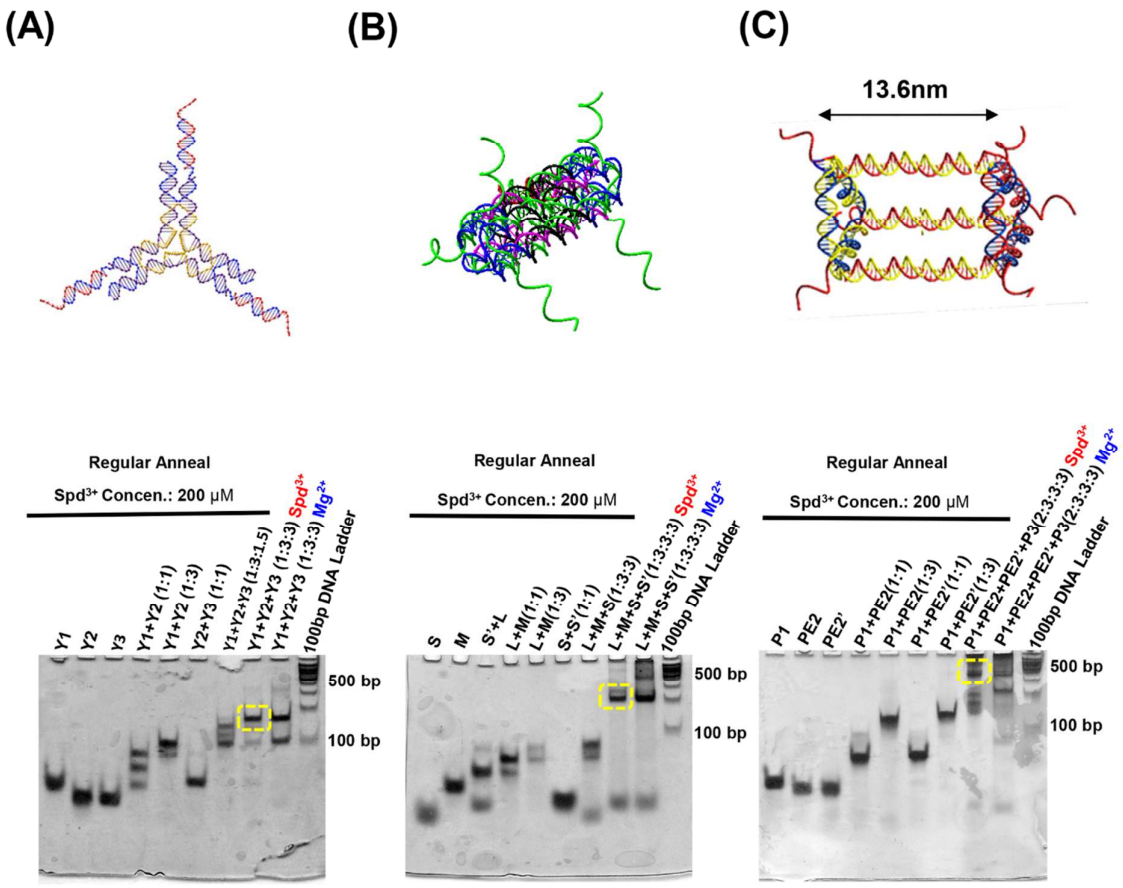

Figure S2 Spermidine-mediated self-assembly of different DNA nanostructures (A) PAGE gel (6%) analysis of a "Shuriken"-like DNA structure (detailed design and sequence infounation can be found in Reference 1).[1] The spermidine concentration used here was 200 μM. The DNA Shuriken was synthesized through a regular annealing process: 95 °C /5 mins, 65 °C /30 mins, 50 °C /30 mins, 37 °C /30 mins and 22 °C /30 mins. (B) PAGE gel (6%) analysis of DNA nanotube (detailed design and sequence infounation can be found in Reference 2).[2] (C) The enlongated DNA nanoprism consists of four DNA strands P1, PE2, PE2' and P3. They were mixed at a molar ratio of 2:3:3:3 and then subject to a regular annealing as in (A). The sequences for the individual strands are: PE2 5'-GCA GTT GAT CCT TTG GAT ACC CTG GTT TAG AGA TTG AAG GAC GGA ATG T ATC AGT GTC ACT CTC GCT TGC-3'; PE2' 5'-CGT TCG CTC TCA CTG TGA CTAT TAG AGA TTG

AAG GAC GGA ATG TT G GTC CCA TAG GTT TCC TAG TTG ACG-3‘; P3 5‘-TAG TCA CAG TGA GAG CGA ACG GCA AGC GAG AGT GAC ACT GAT-3‘.

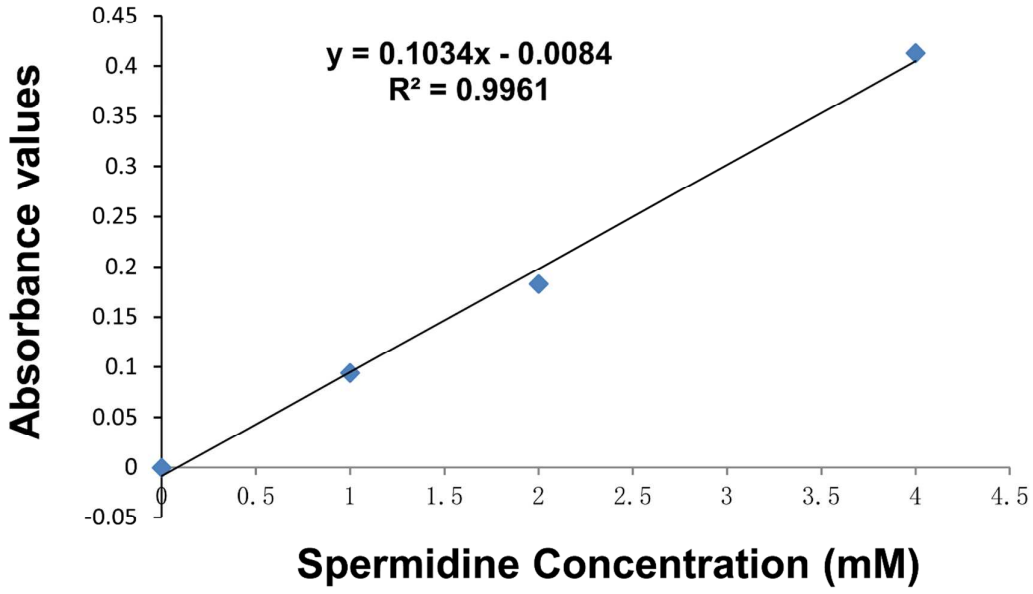

Figure S3 A ninhydrin (2,2-dihydroxyindane-1,3-dione) reagent assay was conducted to determine the remaining spermidine spermidine/DNA nanoprism complex after filtration. Ninhydrin is a chemical used to detect primary and secondary amines. When reacting with these free amines, a deep blue or purple color known as Ruhemann's purple is produced. According to instruction of the ninhydrin kit (Beijing Leagene Biotech.Co., Ltd), the standard curve was firstly established with the serial concentrations of spermidine trihydrochloride solution (0 mM, 1 mM, 2 mM, 4 mM). Spermidine first reacted with ninhydrin working solution and vitamin C solution for 15 mins at 80 ℃, then used the microplate reader to obtain the absorbance at 570 nm. The standard curve was then obtained. Afterwards, the nanoprism solution was filtered by concentrator follow the aforementioned steps and collected the filtered solution to estimate the free spermidine amount. Finally, the spermidine bounded

to DNA was estimated to be 74.4 μM (The original spermidine concentration used for DNA self-assembly is 150 μM)

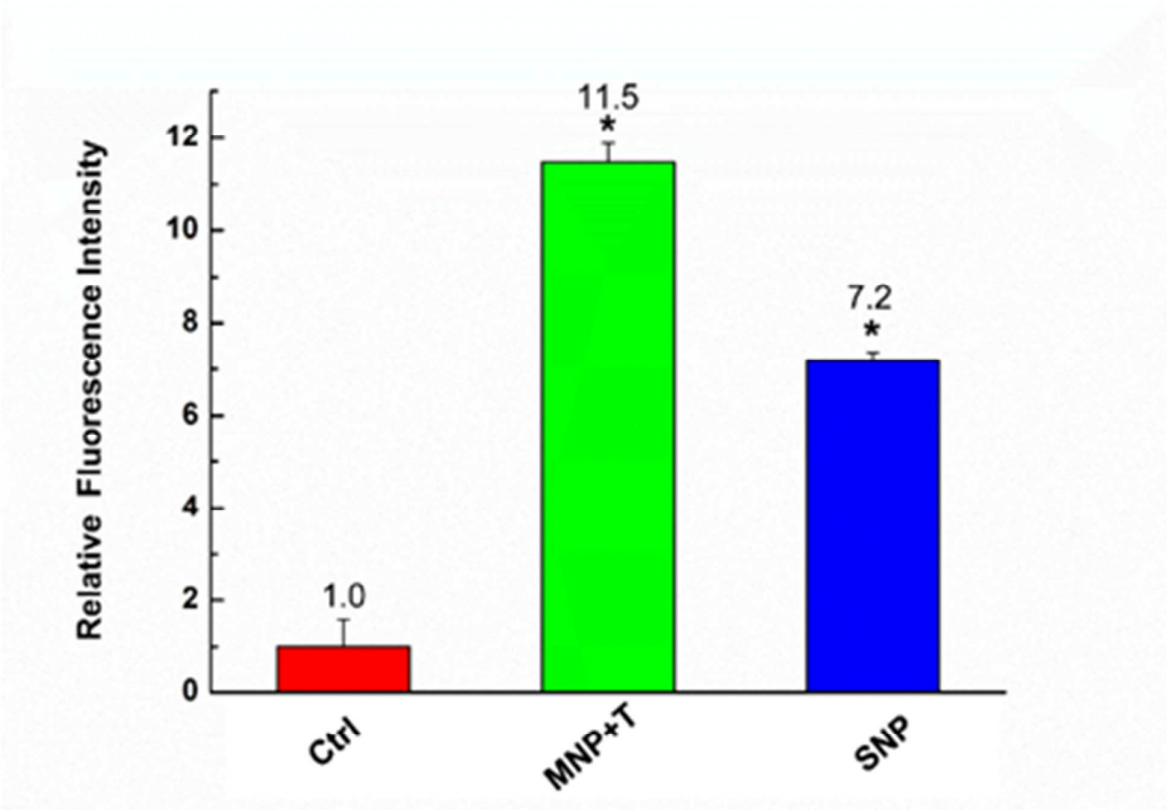

Figure S4 Microplate reader assay that quantifying the cellular uptake efficiencies by measuring the red fluorescence intensities emitted by the internalized DNA nanoprisms. Multiple-point scanning for each well of the 96-well plate was applied to ensure the accuracy. All fluorescence intensity data obtained in this study was normalized to blue fluorescence that emitted from Hoechst stained cell nucleus to eliminate variations resulted from cell density. And the concentration of Cy3 was kept at 300 nM (DNA nanoprism was 150 nM).The MNP+T represented that Mg^{2+} assembled DNA nanoprism was transfencted using Roche X-tremeGENE Transfection Reagent for 12 h and replaced by fresh F-12K medium. And it showed that the absolute internalized SNP amount was significant and comparable with commercial transfection agent transfected MNP.

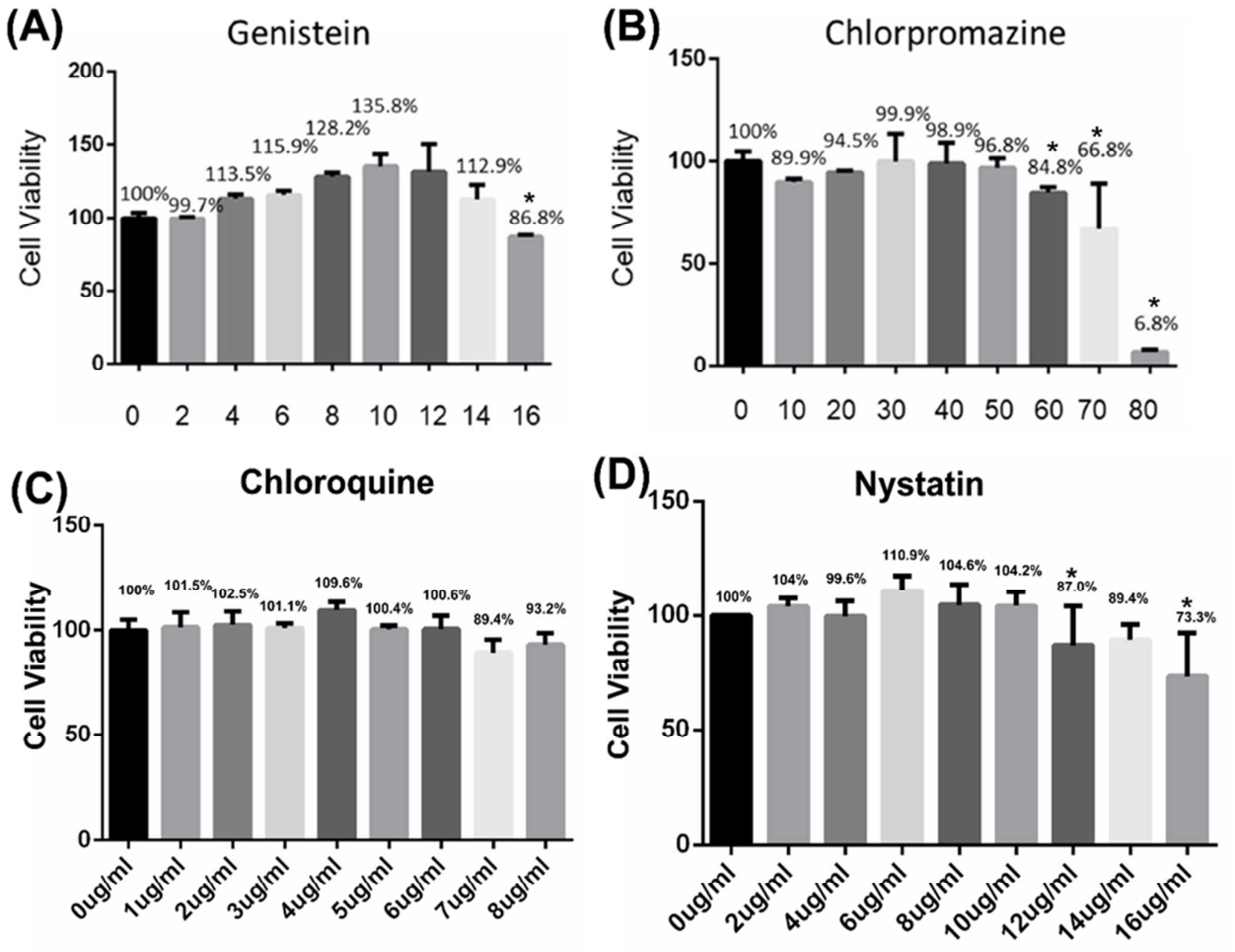

Figure S5 Cytotoxicity of endocytosis inhibitors examined by MTT assay.(A) and (D) The cell viability of A549 cells treated with caveolin inhibitors Genistein and Nystatin. The working concentrations for both inhibitors in current experiment settings were domenstrated to be nontoxic. (B) and (C) The cytotoxicity of clathrin inhibitors estimated by MTT assay indicated that the same conclusion that there was no cytotoxicity of these inhibitors in their working concentration.

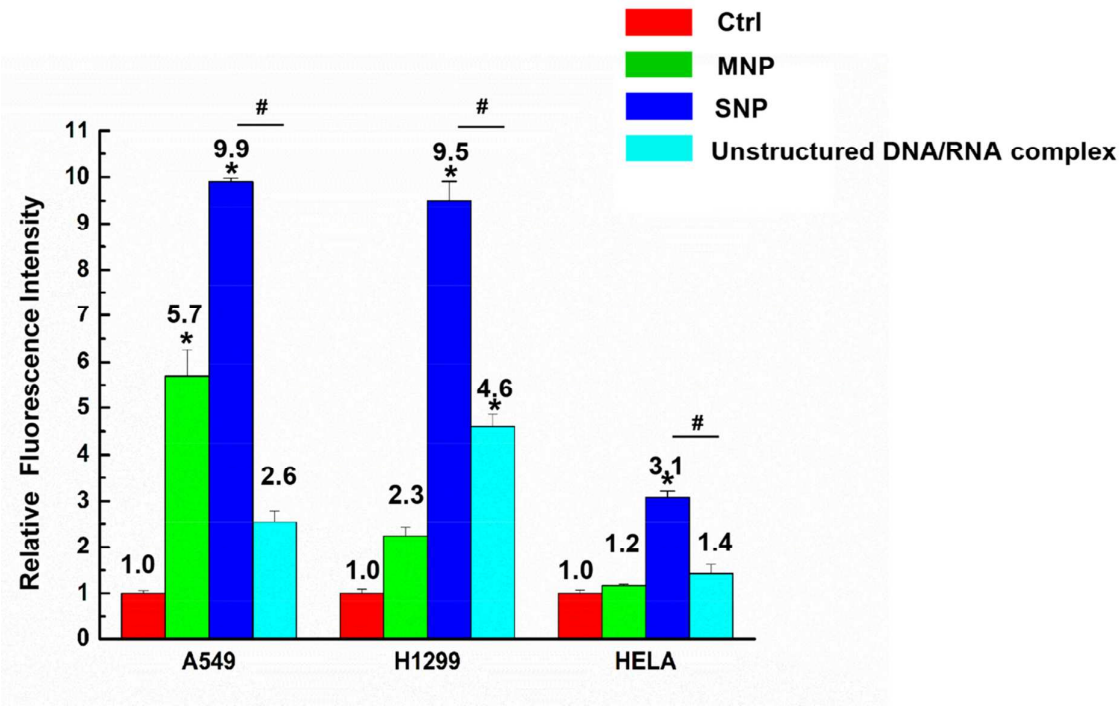

Figure S6 The microplate reader assay quantification of the cellular uptake efficiencies by measuring the red fluorescence intensities emitted by the internalized DNA nanoprisms. Multiple-point scanning for each well of the 96-well plate was applied to ensure accuracy. All fluorescence intensity data obtained in this study were normalized to the blue fluorescence emitted by the Hoechst-stained cell nucleus to eliminate variations resulting from cell density. For all cells, the Cy3 concentration was kept at 300 nM (150 nM DNA nanoprisms). No transfection agents were used in these experiments. The cellular uptake capability of the SNP was evaluated in a series of cancerous (A549, H1299, Hela) cell lines. The MNP and unstructured DNA/siRNA complex were included for all cell lines for comparison. The mean ± S.E., n = 4 Statistical P-values: *P < 0.05, #P < 0.05.

(1) Qian, H.; Tay, C. Y.; Setyawati, M. I.; Chia, S. L.; Lee, D. S.; Leong, D. T. Protecting microRNAs from RNase degradation with steric DNA nanostructures. *Chem Sci* **2017,** *8* (2), 1062-1067, DOI: 10.1039/c6sc01829g.

(2) You, Z.; Qian, H.; Wang, C.; He, B.; Yan, J.; Mao, C.; Wang, G. Regulation of vascular smooth muscle cell autophagy by DNA nanotube-conjugated mTOR siRNA. *Biomaterials* **2015,** *67*, 137-50, DOI: 10.1016/j.biomaterials.2015.07.015.