Electronic Supplementary Material (ESI) for Nanoscale.

SUPPLEMENTARY DATA

Enhanced and Synergistic downregulation of oncogenic miRNAs by self-assembled DNA nanostructures

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Table S1. Sequences of various oligonucleotides used for one-step assembly reaction to generate bDNA nanostructures

Oligos	Sequences
miR-27a	5′ TTCACAGTGGCTAAGTTCCGC 3′
miR-96	5′ TTTGGCACTAGCACATTTTTGCT 3′
miR-182	5′ TTTGGCAATGGTAGAACTCACACT 3′
Scramble	5′ TTCGGAAGAGACAGCG 3′
AntimiR-27a	5′ GCGGAACTTAGCCACTGTGAA 3′
AntimiR-96	5′ AGCAAAAATGTGCTAGTGCCAAA 3′
AntimiR-182	5′ AGTGTGAGTTCTACCATTGCCAAA 3′
I-scramble	5′ TTCGGAAGAGACAGCG TTT CTGAGGAGAGCAGCGCTTGGCCAGCGCCTC TTT TTCGGAAGAGACAGCG 3′
I-antimiR-27a	5′ GCGGAACTTAGCCACTGTGAA TTT CTGAGGAGAGCAGCGCTTGGCCAGCGCCTC TTT GCGGAACTTAGCCACTGTGAA 3′
I-antimiR-96	5′ AGCAAAAATGTGCTAGTGCCAAA TTT CTGAGGAGAGCAGCGCTTGGCCAGCGCCTC TTT AGCAAAAATGTGCTAGTGCCAAAA 3′
I-antimiR-182	5′ AGTGTGAGTTCTACCATTGCCAAA TTT CTGAGGAGAGCAGCGCTTGGCCAGCGCCTC TTT AGTGTGAGTTCTACCATTGCCAAA 3′
I-antimiR-27a-alexa488	5′ GCG GAA CTT AGC CAC TGT GAA TTT CTG AGG AGA GCA GCG CTT GGC CAG CGC CTC TTT GCG GAA CTT AGC CAC TGT GAA-alexa488 3′
D-scramble	5′ TTCGGAAGAGACAGCG TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCAT TTT TCGGAAGAGACAGCG 3′
D-antimiR-27a	5′ GCGGAACTTAGCCACTGTGAA TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCA TTT GCGGAACTTAGCCACTGTGAA 3′
D-antimiR-96	5′ AGCAAAAATGTGCTAGTGCCAAA TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCA TTT AGCAAAAATGTGCTAGTGCCAAA 3′
D-antimiR-182	5′ AGTGTGAGTTCTACCATTGCCAAA TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCA TTT AGTGTGAGTTCTACCATTGCCAAA 3′
D-antimiR-96 and 182	5′ AGCAAAAATGTGCTAGTGCCAAA TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCA TTT AGTGTGAGTTCTACCATTGCCAAA 3′
B	5′ CTGAGT GACGTTGTG TTT GAGGCGCTGGCCAAGCGCTGCTCTCCTCAG TTT CGAATGGAGAGGCAG 3′
C	5′ CTGCCTCTCCATTCG TTT TGAGCAGAAGGCAAGTAGCAGGACAGCAGA TTT CACAACGTCACTCAG 3′

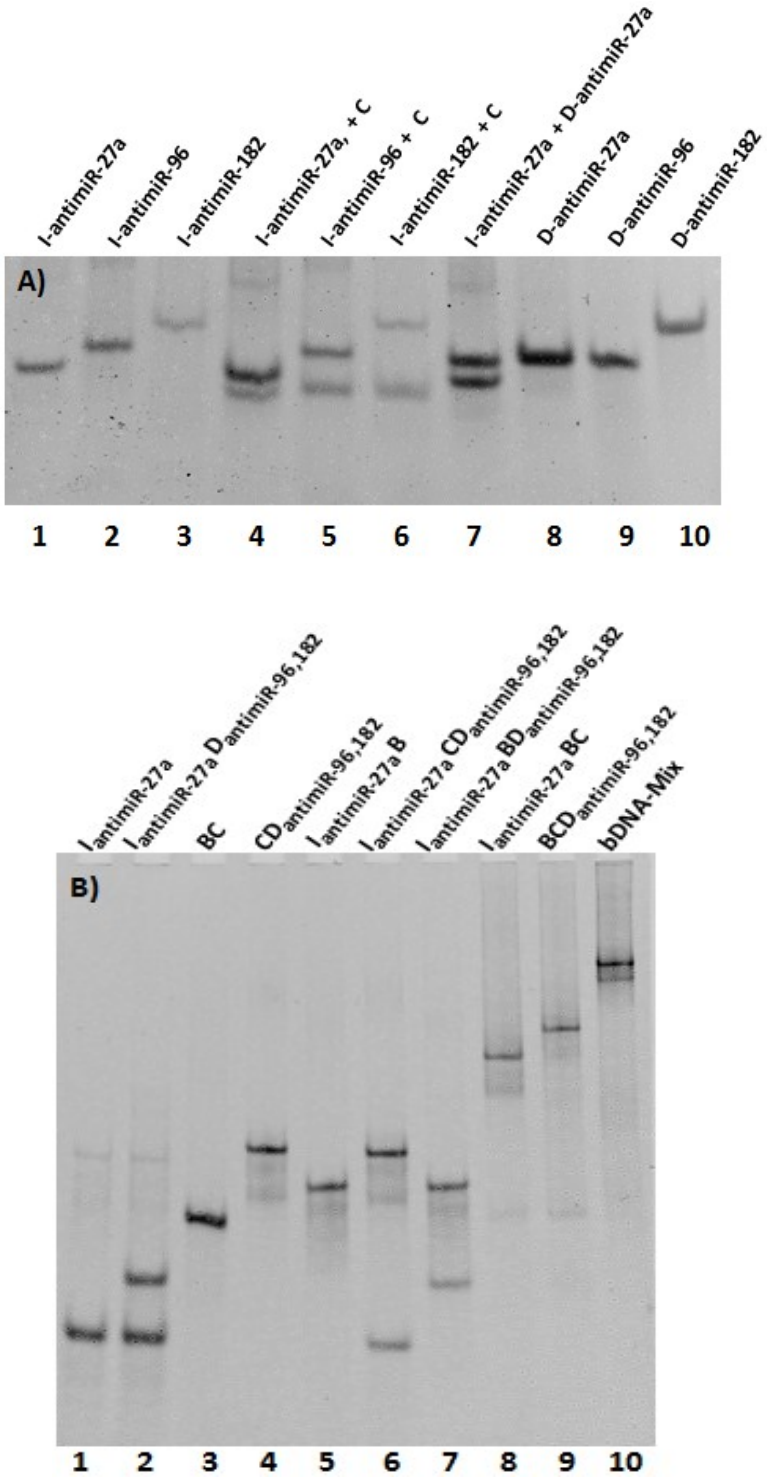

Figure S1. A) Characterization of single oligonucleotides in 10% nPAGE shows no interaction between non-complementary oligos which revealed specificity in oligo designing. B) Characterization of self-assembled bDNA-Mix and its di-oligo and tri-oligo complexes through 10% nPAGE. The sample composition in each lane is mentioned on top of the lane. bDNA-Mix showed decreased electrophoretic mobility with respect to its tri-oligo and di-oligo complexes. Oligos with complementary sequences resulted in desired product where as

oligos with non-complementarity (I-antimiR-27a and D-antimiR-96,182) remained unbound during self-assembly process. Presence of single intense band in each lane showed precise base-pairing among designed oligos.

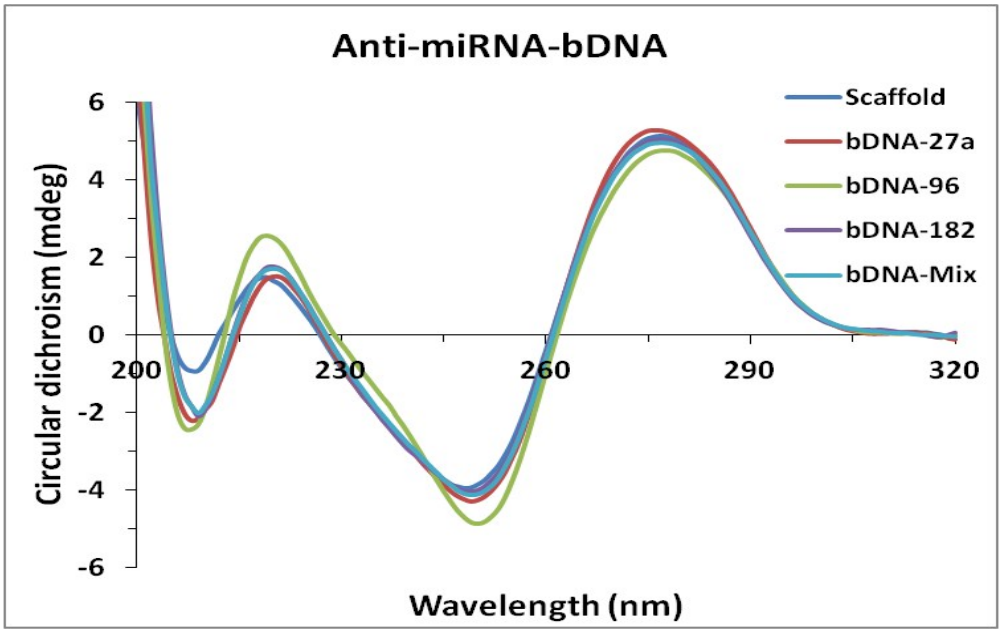

Figure S2. Conformational study of scaffold and bDNA structures. After self-assembly, conformation of all the bDNA structures were observed through CD spectrophotometer. A typical B-conformation of DNA was noticed with characteristic positive peaks at ~280 nm and ~ 220 nm and a negative peak at ~250 nm.

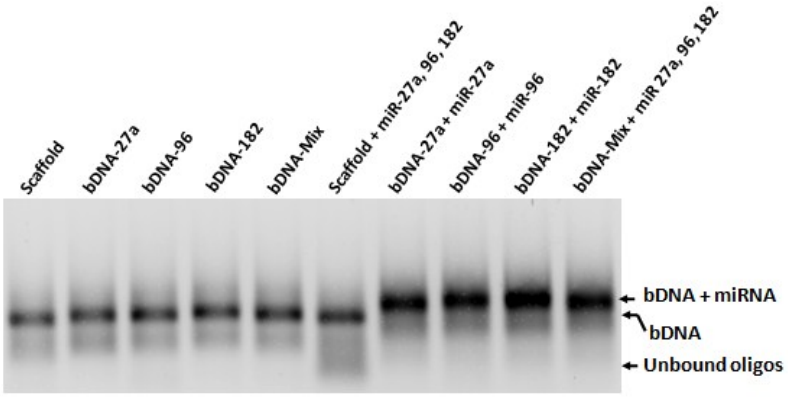

Figure S3: *In vitro* binding study between bDNA structures with complementary miRNA sequences. The self-assembled bDNA structures (bDNA-27a, bDNA-96, bDNA-182 and bDNA-Mix) wihen incubated with miR-27a, miR-96, and/or miR-182, a clear retardation was observed in the electrophoretic mobility (lane 7-10 from left) in comparison to bDNA structures incubated without miRNAs (lanes 1 to 5). No change in migration was observed in scaffold when incubated with miRNA oligos (lane 6).

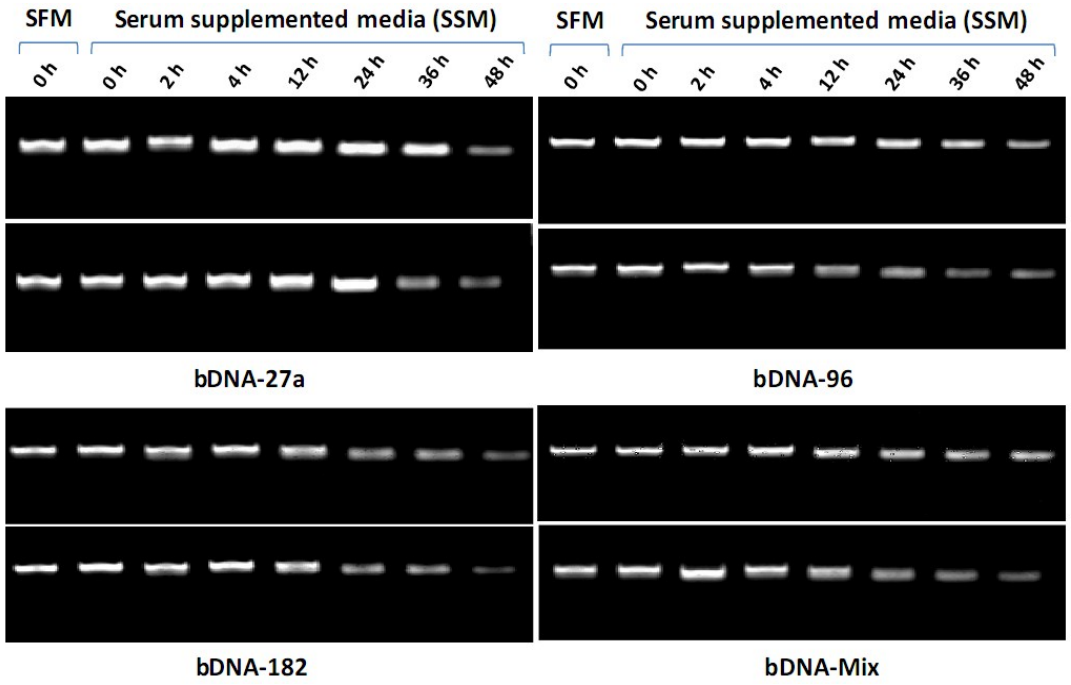

Figure S4. Serum stability study of bDNA structures (bDNA-27a, bDNA-96, bDNA-182 and bDNA-Mix) through agarose gel electrophoresis after incubation at 37°C for 0 to 48 h. (SFM: Serum-free media, SSM: Serum-supplemented media).

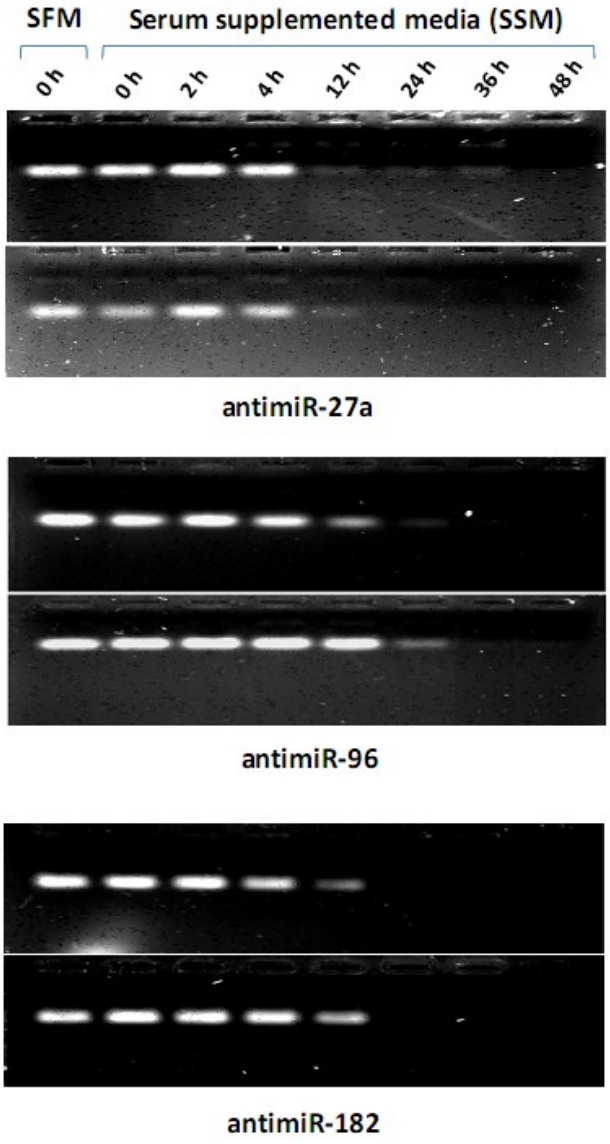

Figure S5. Serum stability study of antimiRNAs (antimiR-27a, 96 and 182) through agarose gel electrophoresis after incubation at 37°C for 0 to 48 h. (SFM: Serum-free media, SSM: Serum-supplemented media).

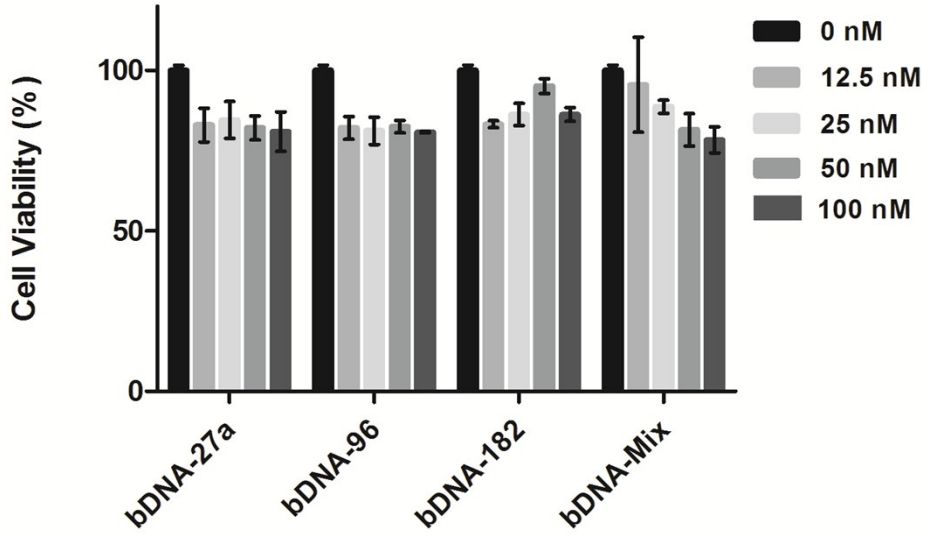

Figure S6: MTT assays of cell viability. bDNA nanostructures were transfected in MCF-7 cells from 0-100 nM and incubated for 24 h. There is no measurable cytotoxicity detected by the bDNA nanostructures. Error bars represent standard deviation (SD) of three independent experiments

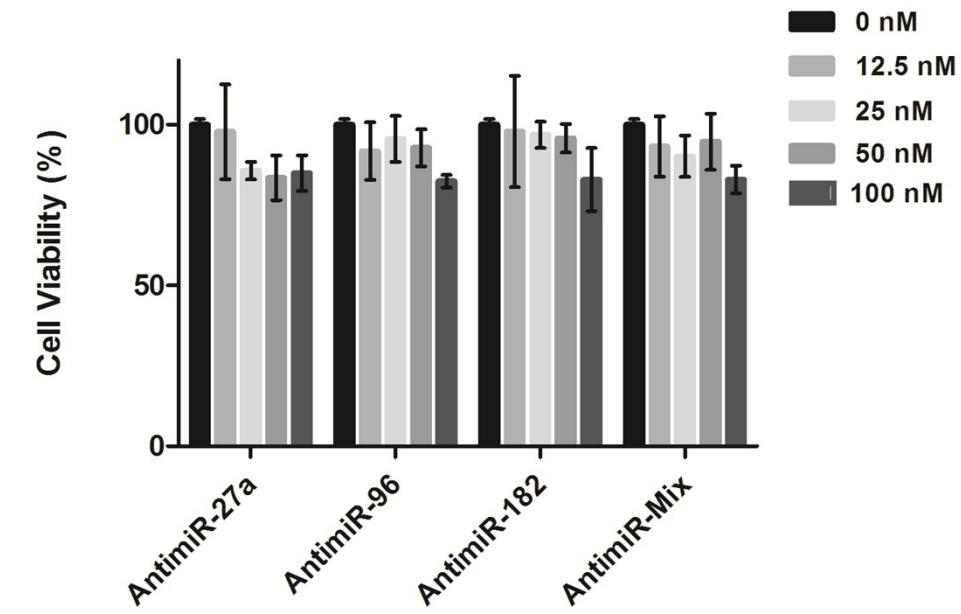

Figure S7: Measuring cell viability by MTT assay of antimiRs in concentration dependent manner from 0-100 nM. Error bars represent standard deviation (SD) of three independent experiments

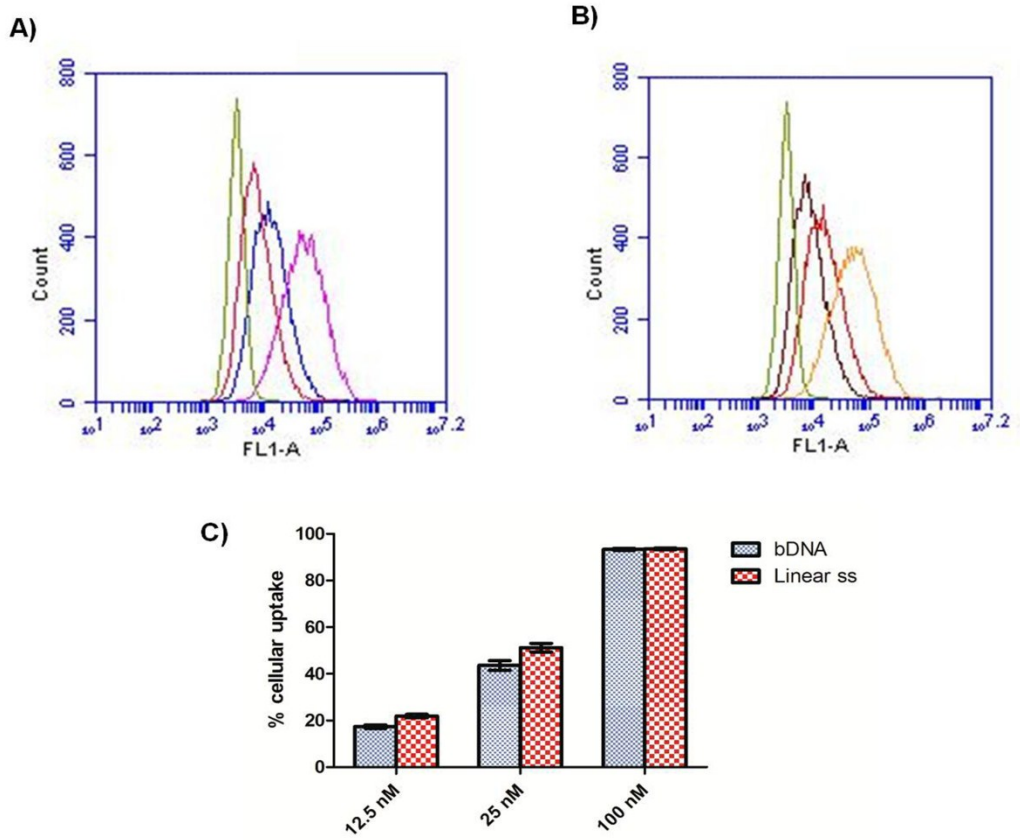

Figure S8: Flow cytometry analysis of transfection efficiency and cellular uptake of bDNA. MCF-7 cells were incubated with increasing concentrations of the Alexa fluor-488 conjugated bDNA-Mix uptake in MCF7 cells was detected by FACS analysis of Alexa-488 labeled cells. (A) Uptake of bDNA-Mix at 12.5 nM (red), 25nM (blue) and 100 nM (pink). Untreated cells are shown in green (B) Uptake of Alexa-488 conugated single stranded oligos. Brown, Red and Orange indicate 12.5 nM, 25 nM and 100 nM respectively. (C) Quantitation of uptake of bDNA nanostructure and its linear single stranded counterpart. The experiment was repeated in triplicate with comparable results.