Electronic Supplementary Material (ESI) for Nanoscale.

Aptamer-tethered DNA Origami Amplifier for Sensitive and Accurate Imaging of Intracellular MicroRNAs

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Chemicals and Materials

TAE/TBE buffer and Acrylamide/bis-acrylamide (30%) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). All oligonucleotides were synthesized and purified using HPLC by Shangya Biotechnology (Fuzhou, China). Dulbecco's Phosphate-Buffered Saline (DPBS), Minimum Essential Medium (MEM), RPMI 1640 medium, penicillin-streptomycin solution (100 mg/mL) and fetal bovine serum (FBS) were obtained from GIBCO (USA). L02, Hela, MCF-7 cell lines were obtained from ATCC (Manassas, VA). Amicon ultra 100 kDa MWCO were purchased from Millipore. Double-distilled ultrapure water (18.2 MΩ cm) was used (Milli-Q, Millipore Corp., Bedford, MA).

Instruments

The assembling of DNA was achieved on a C1000 Touch™ Thermal Cycler (Bio-Rad). Gel electrophoresis images were obtained by a ChemiDoc System from Bio-Rad Laboratories. AFM images were captured using a Mulitmode 8 microscope (Scanasyst in fluid mode, Bruker, Germany). The fluorescence spectra were measured by a Cary Eclipse Fluorimeter (Varian Inc). Confocal microscopy imaging was analyzed with a NIKON-A1 laser-scanning confocal microscopy. Flow cytometric assay was operated using a FACS Canoto flow cytometer (BD Bioscience, U.S.A.). CCK-8 results were obtained from a SH-1000 Lab microplate reader.

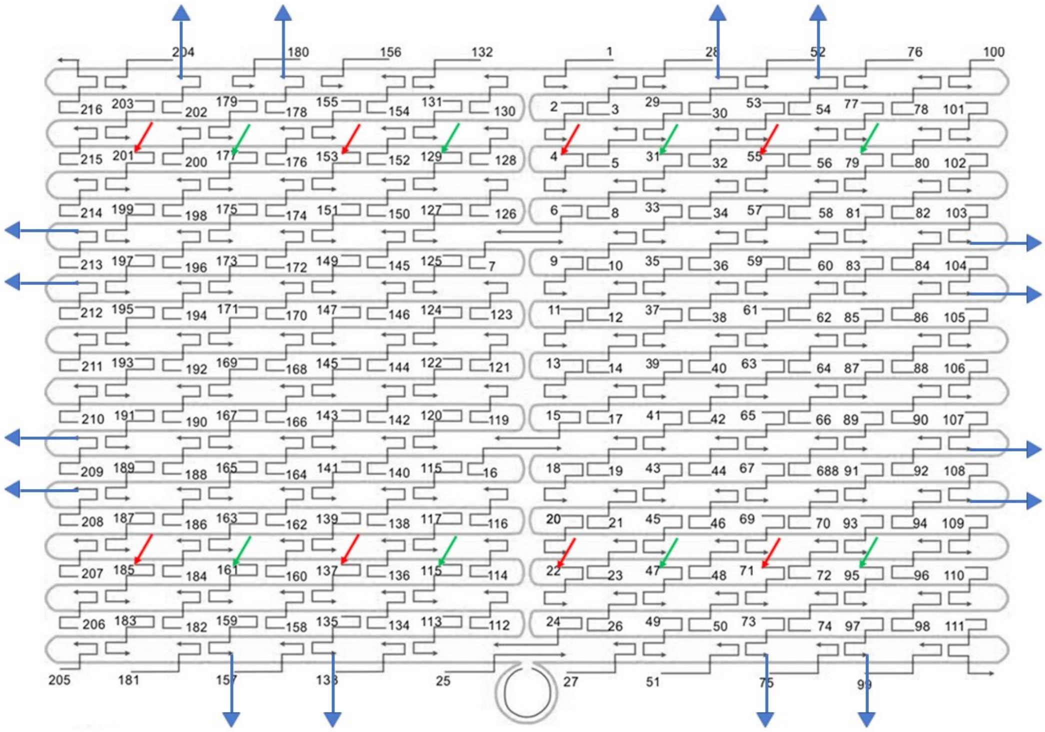

Figure s1. The design of the ADOA structure. M13mp18 single-stranded DNA (gray), staple strands (black) and capture strands (red and green) were fabricated into rectangular origami. Capture strands are extended at the 5’-end with ssDNA composed of binding sites on the surface of rectangular origami. 16 targeting strands (blue) with 3’-end extended AS1411 sequences are placed at the four edges of the rectangle.

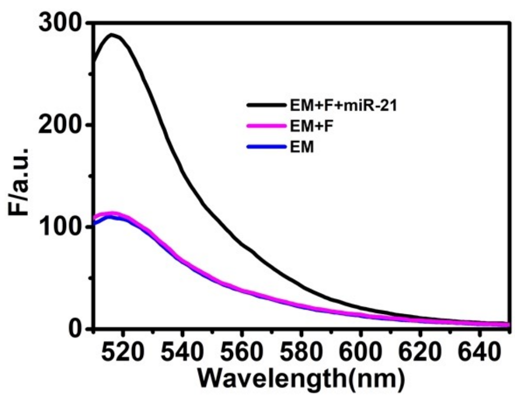

Figure s2. Fluorescence spectra of ADOA respond to target miR-21 in vitro.

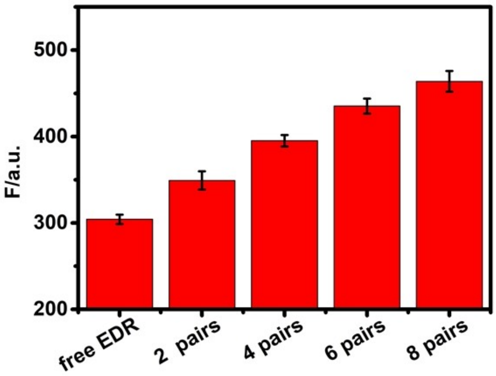

Figure s3. Fluorescence intensity of the free EDR, and 2, 4, 6 pairs of EDR modules self-assembled on DNA origami in response to 10 nM miR-21. Error bars represented variations between three replicate measurements.

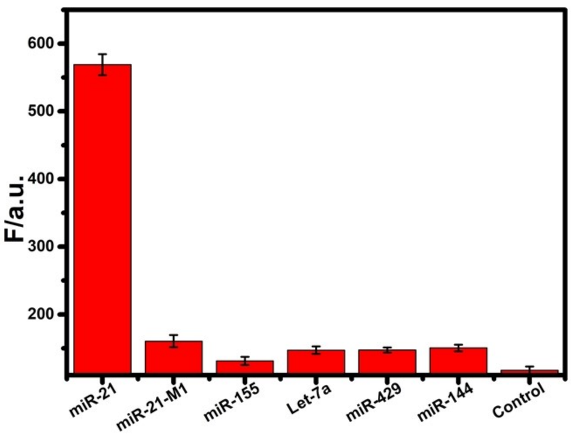

Figure s4. Specificity of the ADOA for several miRNA targets. Error bars were estimated from three replicate measurements.

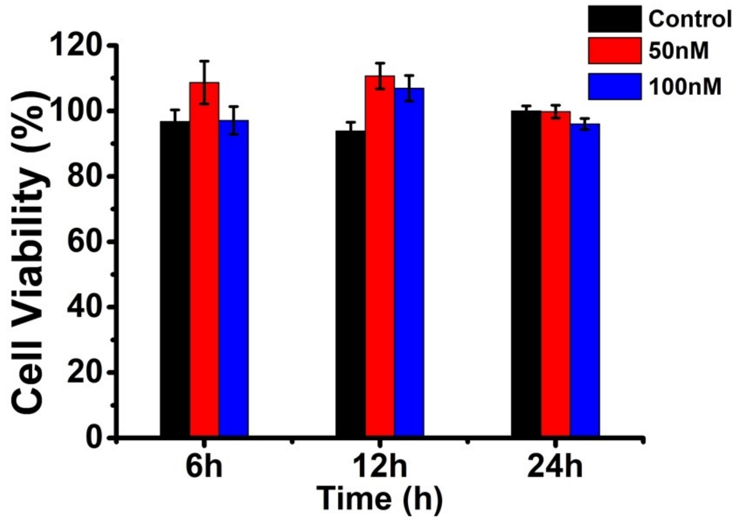

Figure s5. Cytotoxicity of the ADOA incubated with MCF-7 cells at different concentrations of probes.

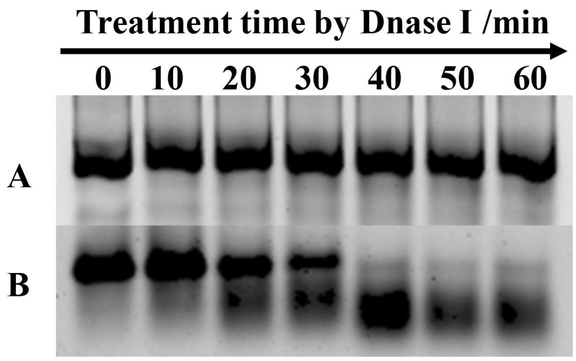

Figure s6. Electrophoresis characterization for the degradation of ADOA (A) and EBs (B) treated with 0.5U/mL Dnase I.

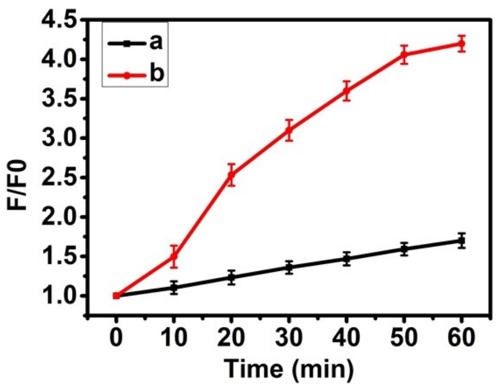

Figure s7. Fluorescence analysis of the degradation of ADOA (a) and EBs (b) treated with 0.5U/mL Dnase I.

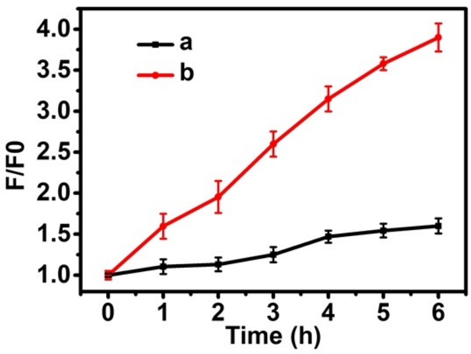

Figure s8. Comparison of nuclease stability of ADOA (a) and EBs (b) in 10% FBS.

The ADOA system is better able to resist the degradation of the DNase I and FBS compared to free EB probes likely because the rigidity, compact organization, and charge density of the origami structure decrease its susceptibility to degradation and propensity to interact with DNase I.

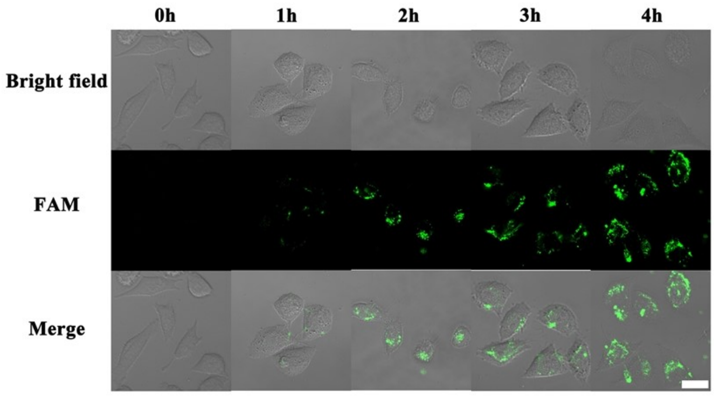

Figure s9. Optimization of incubation time for ADOA in living cells. MCF-7 cells were incubated with ADOA for different time points at 37 °C for confocal microscopy. Scale bars are 20 μm.

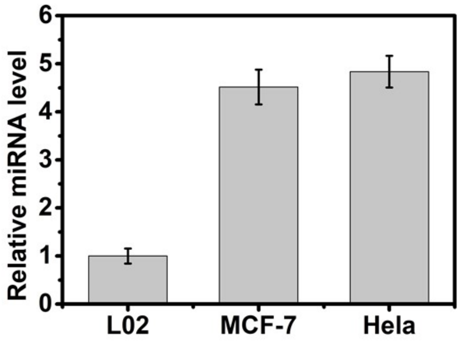

Figure s10. qRT-PCR analysis of relative expression levels of miR-21 in L02 cells, MCF-7 cells and HeLa cells.

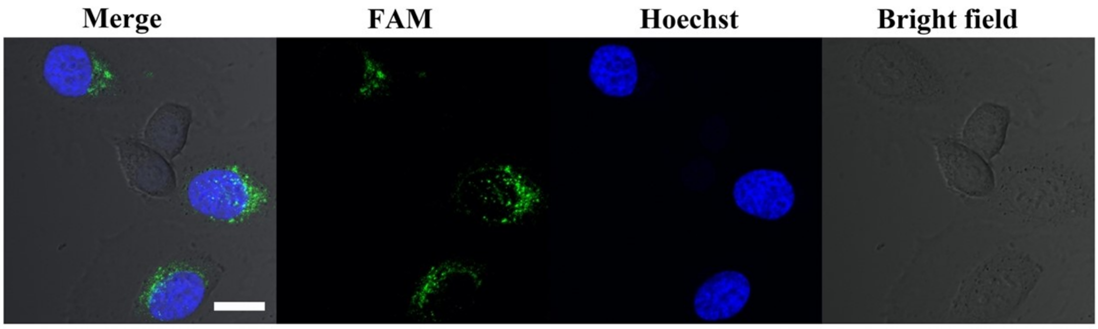

Figure s11. Confocal microscopic images of the co-culture L02 and HeLa cells after being incubated with ADOA. Scare bar: 20 μm.

HeLa cells were first cultured in 1640 medium with 10% fetal calf serum at 37 °C in a 5% CO_2 atmosphere. Then, the cells were incubated with 1 μM Hoechst dye for 10 min and then washed with PBS. Afterward, the above HeLa cells were cultured with L02 cells for 12h. then, the co-cultured HeLa and L02 cells were incubated with ADOA for 4 h. Finally, the cells were washed with PBS before fluorescence imaging.

Table S1. Sequences of the staple strands for the rectangular DNA origami.

Name	Sequence (5'-3')
1	CAAGCCCAATAGGAAC CCATGTACAAACAGTT
2	AATGCCCCGTAACAGT GCCCGTATCTCCCTCA
3	TGCCTTGACTGCCTAT TTCGGAACAGGGATAG
4	GAGCCGCCCCACCACC GGAACCGCGACGGAAA
5	AACCAGAGACCCTCAG AACCGCCAGGGGTCAG

6	TTATTCATAGGGAAGG TAAATATTCATTCAGT
7	CATAACCCGAGGCATA GTAAGAGCTTTTTAAG
8	ATTGAGGGTAAAGGTG AATTATCAATCACCGG
9	AAAAGTAATATCTTAC CGAAGCCCTTCCAGAG
10	GCAATAGCGCAGATAG CCGAACAATTCAACCG
11	CCTAATTTACGCTAAC GAGCGTCTAATCAATA
12	TCTTACCAGCCAGTTA CAAAATAAATGAAATA
13	ATCGGCTGCGAGCATG TAGAAACCTATCATAT
14	CTAATTTATCTTTCCT TATCATTCATCCTGAA
15	GCGTTATAGAAAAAGC CTGTTTAGAAGGCCGG
16	GCTCATTTTCGCATTA AATTTTTGAGCTTAGA
17	AATTACTACAAATTCT TACCAGTAATCCCATC
18	TTAAGACGTTGAAAAC ATAGCGATAACAGTAC
19	TAGAATCCCTGAGAAG AGTCAATAGGAATCAT
20	CTTTTACACAGATGAA TATACAGTAAACAATT
21	TTTAACGTTCGGGAGA AACAATAATTTTCCCT
22	CGACAACTAAGTATTA GACTTTACAATACCGA
23	GGATTTAGCGTATTAA ATCCTTTGTTTTCAGG
24	ACGAACCAAAACATCG CCATTAAATGGTGGTT
25	GAACGTGGCGAGAAAG GAAGGGAACAAACTAT
26	TAGCCCTACCAGCAGA AGATAAAAACATTTGA
27	CGGCCTTGCTGGTAAT ATCCAGAACGAACTGA
28	CTCAGAGCCACCACCC TCATTTTCCTATTATT
29	CTGAAACAGGTAATAA GTTTTAACCCCTCAGA
30	AGTGTACTTGAAAGTA TTAAGAGGCCGCCACC
31	GCCACCACTCTTTTCA TAATCAAACCGTCACC
32	GTTTGCCACCTCAGAG CCGCCACCGATACAGG
33	GACTTGAGAGACAAAA GGGCGACAAGTTACCA
34	AGCGCCAACCATTTGG GAATTAGATTATTAGC
35	GAAGGAAAATAAGAGC AAGAAACAACAGCCAT
36	GCCCAATACCGAGGAA ACGCAATAGGTTTACC
37	ATTATTTAACCCAGCT ACAATTTTCAAGAACG

38	TATTTTGCTCCCAATC CAAATAAGTGAGTTAA
39	GGTATTAAGAACAAGA AAAATAATTAAAGCCA
40	TAAGTCCTACCAAGTA CCGCACTCTTAGTTGC
41	ACGCTCAAAATAAGAA TAAACACCGTGAATTT
42	AGGCGTTACAGTAGGG CTTAATTGACAATAGA
43	ATCAAAATCGTCGCTA TTAATTAACGGATTCG
44	CTGTAAATCATAGGTC TGAGAGACGATAAATA
45	CCTGATTGAAAGAAAT TGCGTAGACCCGAACG
46	ACAGAAATCTTTGAAT ACCAAGTTCCTTGCTT
47	TTATTAATGCCGTCAA TAGATAATCAGAGGTG
48	AGATTAGATTTAAAAG TTTGAGTACACGTAAA
49	AGGCGGTCATTAGTCT TTAATGCGCAATATTA
50	GAATGGCTAGTATTAA CACCGCCTCAACTAAT
51	CCGCCAGCCATTGCAA CAGGAAAAATATTTTT
52	CCCTCAGAACCGCCAC CCTCAGAACTGAGACT
53	CCTCAAGAATACATGG CTTTTGATAGAACCAC
54	TAAGCGTCGAAGGATT AGGATTAGTACCGCCA
55	CACCAGAGTTCGGTCA TAGCCCCCGCCAGCAA
56	TCGGCATTCCGCCGCC AGCATTGACGTTCCAG
57	AATCACCAAATAGAAA ATTCATATATAACGGA
58	TCACAATCGTAGCACC ATTACCATCGTTTTCA
59	ATACCCAAGATAACCC ACAAGAATAAACGATT
60	ATCAGAGAAAGAACTG GCATGATTTTATTTTG
61	TTTTGTTTAAGCCTTA AATCAAGAATCGAGAA
62	AGGTTTTGAACGTCAA AAATGAAAGCGCTAAT
63	CAAGCAAGACGCGCCT GTTTATCAAGAATCGC
64	AATGCAGACCGTTTTT ATTTTCATCTTGCGGG
65	CATATTTAGAAATACC GACCGTGTTACCTTTT
66	AATGGTTTACAACGCC AACATGTAGTTCAGCT
67	TAACCTCCATATGTGA GTGAATAAACAAAATC
68	AAATCAATGGCTTAGG TTGGGTTACTAAATTT
69	GCGCAGAGATATCAAA ATTATTTGACATTATC

70	AACCTACCGCGAATTA TTCATTTCCAGTACAT
71	ATTTTGCGTCTTTAGG AGCACTAAGCAACAGT
72	CTAAAATAGAACAAAG AAACCACCAGGGTTAG
73	GCCACGCTATACGTGG CACAGACAACGCTCAT
74	GCGTAAGAGAGAGCCA GCAGCAAAAAGGTTAT
75	GGAAATACCTACATTT TGACGCTCACCTGAAA
76	TATCACCGTACTCAGG AGGTTTAGCGGGGTTT
77	TGCTCAGTCAGTCTCT GAATTTACCAGGAGGT
78	GGAAAGCGACCAGGCG GATAAGTGAATAGGTG
79	TGAGGCAGGCGTCAGA CTGTAGCGTAGCAAGG
80	TGCCTTTAGTCAGACG ATTGGCCTGCCAGAAT
81	CCGGAAACACACCACG GAATAAGTAAGACTCC
82	ACGCAAAGGTCACCAA TGAAACCAATCAAGTT
83	TTATTACGGTCAGAGG GTAATTGAATAGCAGC
84	TGAACAAACAGTATGT TAGCAAACTAAAAGAA
85	CTTTACAGTTAGCGAA CCTCCCGACGTAGGAA
86	GAGGCGTTAGAGAATA ACATAAAAGAACACCC
87	TCATTACCCGACAATA AACAACATATTTAGGC
88	CCAGACGAGCGCCCAA TAGCAAGCAAGAACGC
89	AGAGGCATAATTTCAT CTTCTGACTATAACTA
90	TTTTAGTTTTTCGAGC CAGTAATAAATTCTGT
91	TATGTAAACCTTTTTT AATGGAAAAATTACCT
92	TTGAATTATGCTGATG CAAATCCACAAATATA
93	GAGCAAAAACTTCTGA ATAATGGAAGAAGGAG
94	TGGATTATGAAGATGA TGAAACAAAATTTCAT
95	CGGAATTATTGAAAGG AATTGAGGTGAAAAAT
96	ATCAACAGTCATCATA TTCCTGATTGATTGTT
97	CTAAAGCAAGATAGAA CCCTTCTGAATCGTCT
98	GCCAACAGTCACCTTG CTGAACCTGTTGGCAA
99	GAAATGGATTATTTAC ATTGGCAGACATTCTG
100	TTTT TATAAGTA TAGCCCGGCCGTCGAGAGGGTTGA
101	TTTT ATAAATCC TCATTAAATGATATTCACAAACAA

102	TTTT AATCAGTA GCGACAGATCGATAGCAGCACCGT
103	TTTT TAAAGGTG GCAACATAGTAGAAAATACATACA
104	TTTT GACGGGAG AATTAACTACAGGGAAGCGCATTA
105	TTTT GCTTATCC GGTATTCTAAATCAGATATAGAAG
106	TTTT CGACAAAA GGTAAAGTAGAGAATATAAAGTAC
107	TTTT CGCGAGAA AACTTTTTATCGCAAGACAAAGAA
108	TTTT ATTAATTA CATTTAACACATCAAGAAAACAAA
109	TTTT TTCATCAA TATAATCCTATCAGATGATGGCAA
110	TTTT AATCAATA TCTGGTCACAAATATCAAACCCTC
111	TTTT ACCAGTAA TAAAAGGGATTCACCA GTCACACG TTTT
112	CCGAAATCCGAAAATC CTGTTTGAAGCCGGAA
113	CCAGCAGGGGCAAAAT CCCTTATAAAGCCGGC
114	GCATAAAGTTCCACAC AACATACGAAGCGCCA
115	GCTCACAATGTAAAGC CTGGGGTGGGTTTGCC
116	TTCGCCATTGCCGGAA ACCAGGCATTAAATCA
117	GCTTCTGGTCAGGCTG CGCAACTGTGTTATCC
118	GTTAAAATTTTAACCA ATAGGAACCCGGCACC
119	AGACAGTCATTCAAAA GGGTGAGAAGCTATAT
120	AGGTAAAGAAATCACC ATCAATATAATATTTT
121	TTTCATTTGGTCAATA ACCTGTTTATATCGCG
122	TCGCAAATGGGGCGCG AGCTGAAATAATGTGT
123	TTTTAATTGCCCGAAA GACTTCAAAACACTAT
124	AAGAGGAACGAGCTTC AAAGCGAAGATACATT
125	GGAATTACTCGTTTAC CAGACGACAAAAGATT
126	GAATAAGGACGTAACA AAGCTGCTCTAAAACA
127	CCAAATCACTTGCCCT GACGAGAACGCCAAAA
128	CTCATCTTGAGGCAAA AGAATACAGTGAATTT
129	AAACGAAATGACCCCC AGCGATTATTCATTAC
130	CTTAAACATCAGCTTG CTTTCGAGCGTAACAC
131	TCGGTTTAGCTTGATA CCGATAGTCCAACCTA
132	TGAGTTTCGTCACCAG TACAAACTTAATTGTA
133	CCCCGATTTAGAGCTT GACGGGGAAATCAAAA

134	GAATAGCCGCAAGCGG TCCACGCTCCTAATGA
135	GAGTTGCACGAGATAG GGTTGAGTAAGGGAGC
136	GTGAGCTAGTTTCCTG TGTGAAATTTGGGAAG
137	TCATAGCTACTCACAT TAATTGCGCCCTGAGA
138	GGCGATCGCACTCCAG CCAGCTTTGCCATCAA
139	GAAGATCGGTGCGGGC CTCTTCGCAATCATGG
140	AAATAATTTTAAATTG TAAACGTTGATATTCA
141	GCAAATATCGCGTCTG GCCTTCCTGGCCTCAG
142	ACCGTTCTAAATGCAA TGCCTGAGAGGTGGCA
143	TATATTTTAGCTGATA AATTAATGTTGTATAA
144	TCAATTCTTTTAGTTT GACCATTACCAGACCG
145	CGAGTAGAACTAATAG TAGTAGCAAACCCTCA
146	GAAGCAAAAAAGCGGA TTGCATCAGATAAAAA
147	TCAGAAGCCTCCAACA GGTCAGGATCTGCGAA
148	CCAAAATATAATGCAG ATACATAAACACCAGA
149	CATTCAACGCGAGAGG CTTTTGCATATTATAG
150	ACGAGTAGTGACAAGA ACCGGATATACCAAGC
151	AGTAATCTTAAATTGG GCTTGAGAGAATACCA
152	GCGAAACATGCCACTA CGAAGGCATGCGCCGA
153	ATACGTAAAAGTACAA CGGAGATTTCATCAAG
154	CAATGACACTCCAAAA GGAGCCTTACAACGCC
155	AAAAAAGGACAACCAT CGCCCACGCGGGTAAA
156	TGTAGCATTCCACAGA CAGCCCTCATCTCCAA
157	GTAAAGCACTAAATCG GAACCCTAGTTGTTCC
158	AGTTTGGAGCCCTTCA CCGCCTGGTTGCGCTC
159	AGCTGATTACAAGAGT CCACTATTGAGGTGCC
160	ACTGCCCGCCGAGCTC GAATTCGTTATTACGC
161	CCCGGGTACTTTCCAG TCGGGAAACGGGCAAC
162	CAGCTGGCGGACGACG ACAGTATCGTAGCCAG
163	GTTTGAGGGAAAGGGG GATGTGCTAGAGGATC
164	CTTTCATCCCCAAAAA CAGGAAGACCGGAGAG
165	AGAAAAGCAACATTAA ATGTGAGCATCTGCCA

166	GGTAGCTAGGATAAAA ATTTTTAGTTAACATC
167	CAACGCAATTTTTGAG AGATCTACTGATAATC
168	CAATAAATACAGTTGA TTCCCAATTTAGAGAG
169	TCCATATACATACAGG CAAGGCAACTTTATTT
170	TACCTTTAAGGTCTTT ACCCTGACAAAGAAGT
171	CAAAAATCATTGCTCC TTTTGATAAGTTTCAT
172	TTTGCCAGATCAGTTG AGATTTAGTGGTTTAA
173	AAAGATTCAGGGGGTA ATAGTAAACCATAAAT
174	TTTCAACTATAGGCTG GCTGACCTTGTATCAT
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180	CGTAACGATCTAAAGT TTTGTCGTGAATTGCG
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182	TGGACTCCCTTTTCAC CAGTGAGACCTGTCGT
183	TGGTTTTTAACGTCAA AGGGCGAAGAACCATC
184	GCCAGCTGCCTGCAGG TCGACTCTGCAAGGCG
185	CTTGCATGCATTAATG AATCGGCCCGCCAGGG
186	ATTAAGTTCGCATCGT AACCGTGCGAGTAACA
187	TAGATGGGGGGTAACG CCAGGGTTGTGCCAAG
188	ACCCGTCGTCATATGT ACCCCGGTAAAGGCTA
189	CATGTCAAGATTCTCC GTGGGAACCGTTGGTG
190	TCAGGTCACTTTTGCG GGAGAAGCAGAATTAG
191	CTGTAATATTGCCTGA GAGTCTGGAAAACTAG
192	CAAAATTAAAGTACGG TGTCTGGAAGAGGTCA
193	TGCAACTAAGCAATAA AGCCTCAGTTATGACC
194	TTTTTGCGCAGAAAAC GAGAATGAATGTTTAG
195	AAACAGTTGATGGCTT AGAGCTTATTTAAATA
196	ACTGGATAACGGAACA ACATTATTACCTTATG
197	ACGAACTAGCGTCCAA TACTGCGGAATGCTTT

198	CGATTTTAGAGGACAG ATGAACGGCGCGACCT
199	CTTTGAAAAGAACTGG CTCATTATTTAATAAA
200	GCTCCATGAGAGGCTT TGAGGACTAGGGAGTT
201	ACGGCTACTTACTTAG CCGGAACGCTGACCAA
202	AAAGGCCGAAAGGAAC AACTAAAGCTTTCCAG
203	GAGAATAGCTTTTGCG GGATCGTCGGGTAGCA
204	ACGTTAGTAAATGAAT TTTCTGTAAGCGGAGT
205	TTTT CGATGGCC CACTACGTAAACCGTC TATCAGGG
206	TTTT CGGTTTGC GTATTGGGAACGCGCG GGGAGAGG
207	TTTT TGTAAAAC GACGGCCATTCCCAGT CACGACGT
208	TTTT GTAATGGG ATAGGTCAAAACGGCG GATTGACC
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213	TTTT GGAAGAAA AATCTACGACCAGTCA GGACGTTG
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215	TTTT GACAGCAT CGGAACGAACCCTCAG CAGCGAAA
216	TTTT AACTTTCA ACAGTTTCTGGGATTT TGCTAAAC TTTT
Loop 1	AACATCACTTGCCTGAGTAGAAGAACT
Loop 2	TGTAGCAATACTTCTTTGATTAGTAAT
Loop 3	AGTCTGTCCATCACGCAAATTAACCGT
Loop 4	ATAATCAGTGAGGCCACCGAGTAAAAG
Loop 5	ACGCCAGAATCCTGAGAAGTGTTTTT
Loop 6	TTAAAGGGATTTTAGACAGGAACGGT
Loop 7	AGAGCGGGAGCTAAACAGGAGGCCGA
Loop 8	TATAACGTGCTTTCCTCGTTAGAATC
Loop 9	GTACTATGGTTGCTTTGACGAGCACG
Loop 10	GCGCTTAATGCGCCGCTACAGGGCGC

Table S2. Sequences of EDR, the modified staple strands and other oligonucleotides.

Name	Sequence (5’-3’)
E1	TCAACATCAGTCTGATAAGCTAAGGGCCGTAAGAGAGCTGTAGATTGGATCG
F	CGATCCAATCTACAGCTCTCTTACGGCCCTTAGCTTATCAGACTGA
E2	CCACATACATCATATTCCCTTAGCTTATCAGACTGA
E3	CAGTCACTCGATCCAATCTACAGCTCTCTTACGG
Linker-E1	GATTAATCCTGTTCAACATCAGTCTGATAAGCTAAGGGCCGTAAGAGAGCTGTAGATTGGATCG
Lingker-F	AATAATCTCGAGCGATCCAATCTACAGCTCTCTTACGGCCCTTAGCTTATCAGACTGA
201-E1	ACAGGATTAATCACGGCTACTTACTTAGCCGGAACGCTGACCAA
153-E1	ACAGGATTAATCATACGTAAAAGTACAACGGAGATTTCATCAAG
4-E1	ACAGGATTAATCGAGCCGCCCCACCACCGGAACCGCGACGGAAA
55-E1	ACAGGATTAATCCACCAGAGTTCGGTCATAGCCCCCGCCAGCAA
185-E1	ACAGGATTAATCCTTGCATGCATTAATGAATCGGCCCGCCAGGG
137-E1	ACAGGATTAATCTCATAGCTACTCACATTAATTGCGCCCTGAGA
22-E1	ACAGGATTAATCCGACAACTAAGTATTAGACTTTACAATACCGA
71-E1	ACAGGATTAATCATTTTGCGTCTTTAGGAGCACTAAGCAACAGT
177-E2	CTCGAGATTATTTTCATGAAAATTGTGTCGAAATCTGTACAGA
129-E2	CTCGAGATTATTAAACGAAATGACCCCCAGCGATTATTCATTAC
31-E2	CTCGAGATTATTGCCACCACTCTTTTCATAATCAAACCGTCACC
79-E2	CTCGAGATTATTTGAGGCAGGCGTCAGACTGTAGCGTAGCAAGG
161-E2	CTCGAGATTATTCCCGGGTACTTTCCAGTCGGGAAACGGGCAAC
115-E2	CTCGAGATTATTGCTCACAATGTAAAGCCTGGGGTGGGTTTGCC
47-E2	CTCGAGATTATTTTATTAATGCCGTCAATAGATAATCAGAGGTG
95-E2	CTCGAGATTATTCGGAATTATTGAAAGGAATTGAGGTGAAAAAT
miR-21 inhibitor	**UCA**ACAUCAGUCUGAUAAG**CUA**
miR-21 mimic	**UAG**CUUAUCAGACUGAUGU**UGA**
miR-21	UAGCUUAUCAGACUGAUGUUGA
target DNA	TAGCTTATCAGACTGATGTTGA
miR-155	UUAAUGCUAAUCGUGAUAGGGGU

miR-429	UAAUACUGUCUGGUAAAACCGU
miR-144	UACAGUAUAGAUGAUGUACU
let-7a	UGAGGUAGUAGGUUGUAUAGUU
miR-21 RT-primer	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAACATCA

The red Letters represent E1 complementation region. The blue letters represent F complementation region. The green Letters indicate phosphorothioate modification.