# Electronic Supplementary Information (ESI)

# Dual-MicroRNA-Controlled Double-Amplified Cascaded Logic DNA Circuits for Cell Subtypes Accurate Discrimination

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## Supporting Tables:

**Table S1.** Oligonucleotide sequences

| Oligo | Sequences (5'-3') |
|---|---|
| (1) | TGTTTGATCACGCATCCGAGCCGGTCGAAATAGCTTA |
| (2) | GCGTGATCAAACACCATTGTCAC |
| (3) | UGGAGUGUGACAAUGGUGUUUG |
| (4) | CGCACTAATCAGACTGATGTTGACAAAGTTCAACATCAGTCTGATAAGCTATrAGTGCGTGAT |
| (5) | CGCACTAATCAGACTGATGTTGACAAAGTTCAACATCAGTCTGATAAGCTAT |
| (6) | UAGCUUAUCAGACUGAUGUUGA |
| (7) | ACTTTGTCAACATCAGTCTGATTAGCTTATCAGACTGATGTTGA |
| (8) | AGCTATrAGTGCGTGAT |
| Anti-miR-122 | C*A*A*ACACCAUUGUCACACUC*C*A* |
| Anti-miR-21 | U*C*A*ACAUCAGUCUGAUAAGC*U*A* |
| SM miR-21 | UAACUUAUCAGACUGAUGUUGA |
| SM miR-122 | UGGAGUGUGAGAAUGGUGUUUG |
| miR-21 forward primer | ACACTCCAGCTGGGTAGCTTATCAGACTG |
| miR-21 reverse primer | CTCAACTGGTGTCGTGGAGTCGGCAATTCAGT |
| miR-122 forward primer | GCCGTGGAGTGTGACAATGGT |
| miR-122 reverse primer | GTGCAGGGTCCGAGGT |

The rA denotes RNA base, the * represents phosphorothioate modification.

**Supporting Figures:**

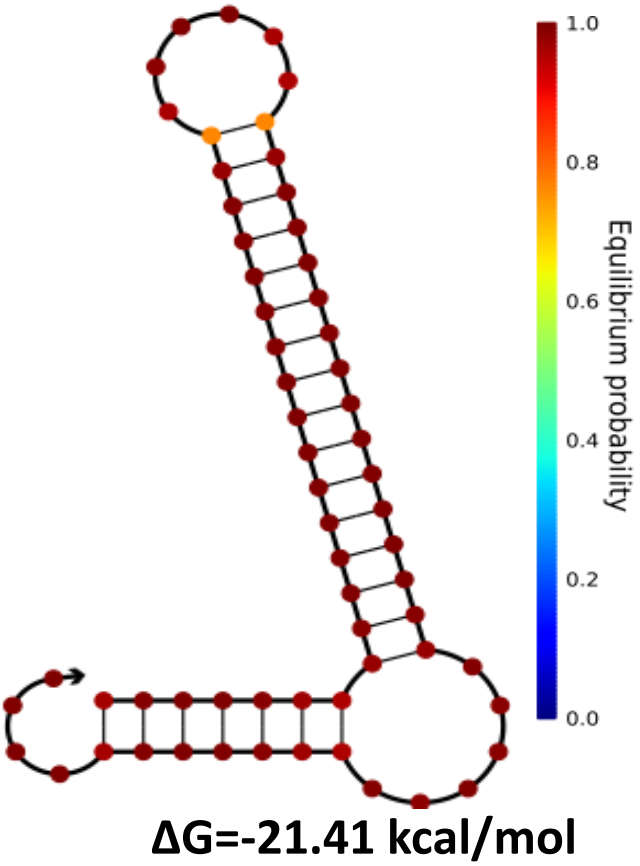


**Figure S1. Analyze of the secondary structure of (4) by NUPACK software.** The hairpin consists of an outer stem and loop, which makes up the upstream DNAzyme binding domain, and an inner stem and loop, which sequester a downstream activator sequence.

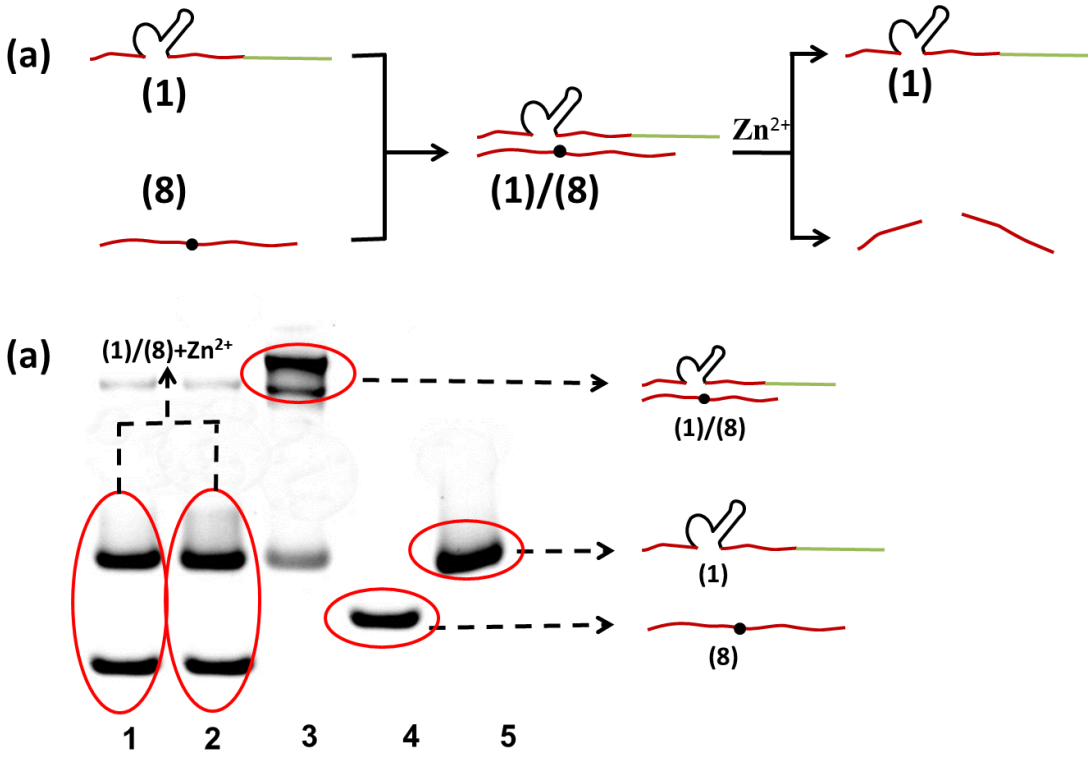


**Figure S2. Proof of the activity of 8-17 DNAzyme.** (a) The scheme of the substrate (8) cleaved by 8-17 DNAzyme. (b) Analysis by 12% PAGE: 1. (1) + (8) + 1 mM $Zn^{2+}$; 2. (1) + (8) + 2 mM $Zn^{2+}$; 3. (1) + (8); 4. (8); 5. (1).

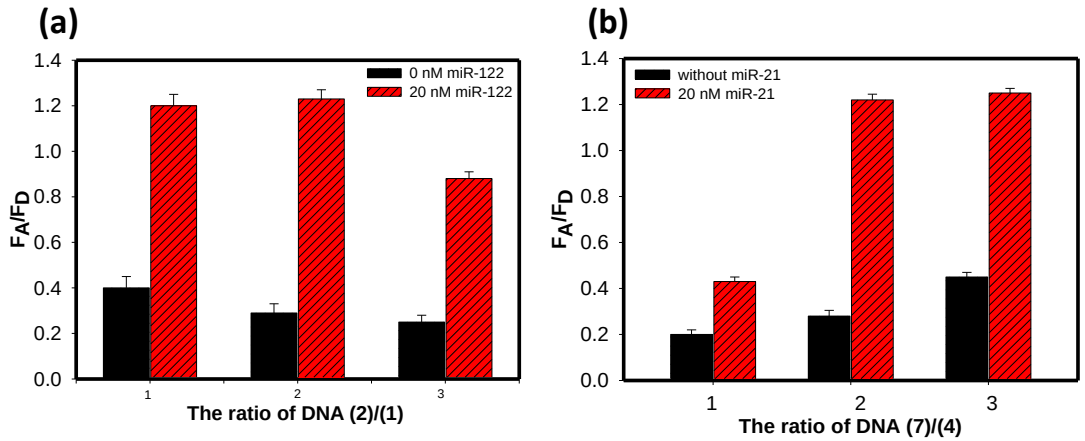


**Figure S3. The optimization of the concentrations of probes. (a)** The fluorescence ratio $F_A/F_D$ under different concentration ratios of DNA (2) and (1) in the presence or absence of 20 nM miR-122. The concentration of (1) is fixed as 100 nM. The error bar was obtained from three independent measurements. **(b)** The fluorescence ratio $F_A/F_D$ under different concentration ratios of hairpin (7) and (4) in the presence or absence of 20 nM miR-21. The concentration of (4) is fixed as 100 nM.

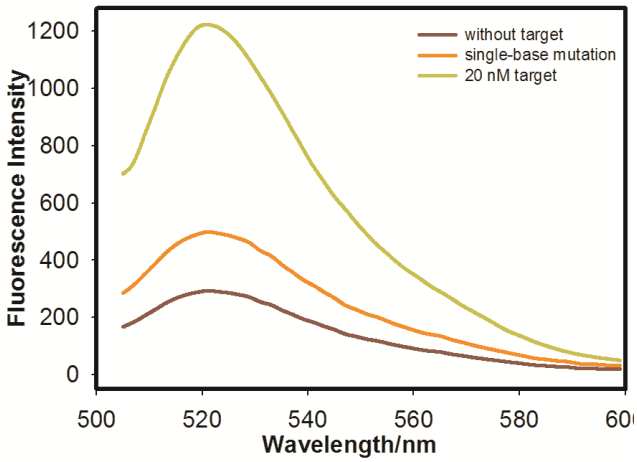


**Figure S4. Selectivity analysis of the signal-activatable DNAzyme amplified circuit for target miR-122.** Both of the miR-122 and single-base mutation DNA were 20 nM.

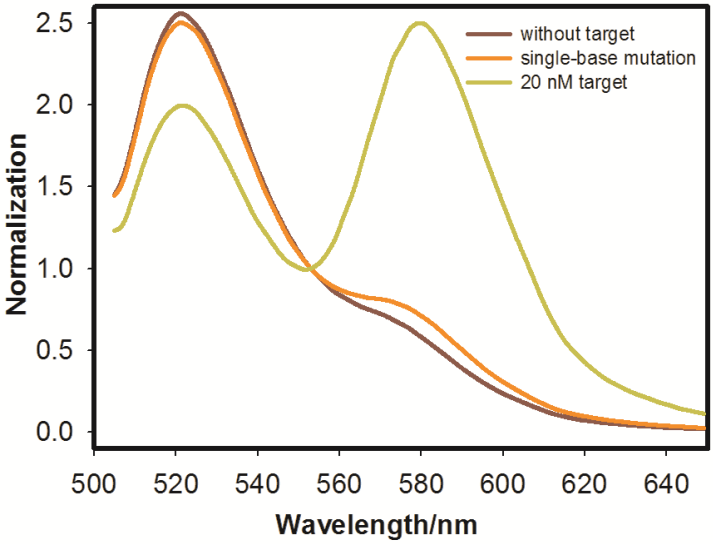


**Figure S5. Selectivity analysis of FRET-based HCR amplified circuit for miR-21.** Both of the miR-21 and single-base mutation DNA were 20 nM.

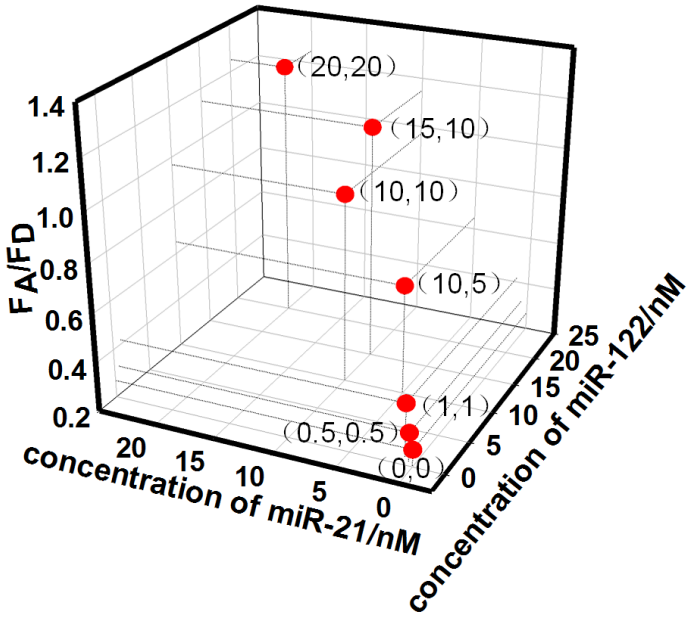


**Figure S6. The $F_A/F_D$ ratios respond to both the concentrations of miR-21 and miR-122.** The values in parentheses represent the concentration of miR-122 **(3)** and miR-21 **(6)** respectively.

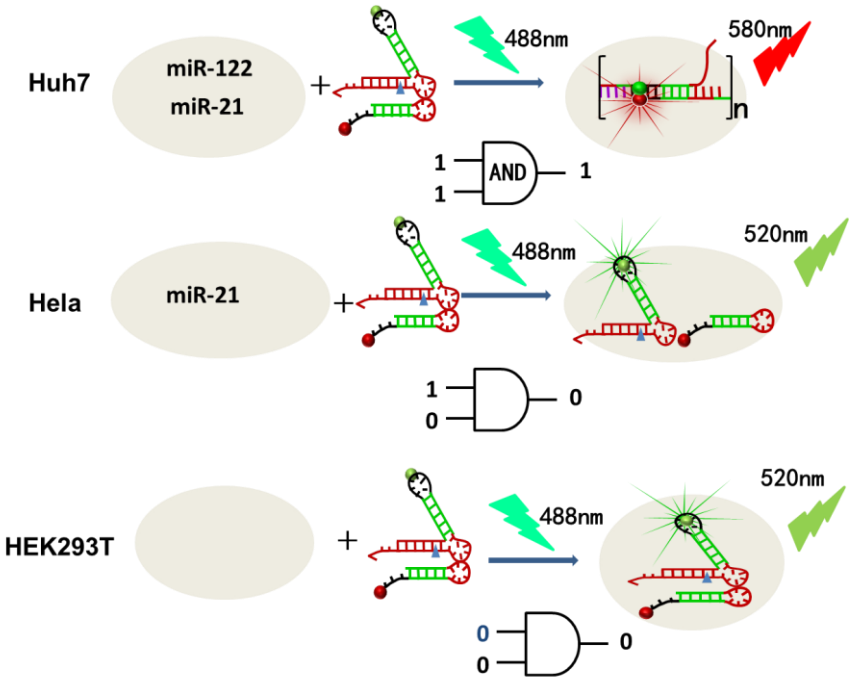


**Figure S7. The logical gate operation of the cascaded DNA circuits with the three cell lines.**

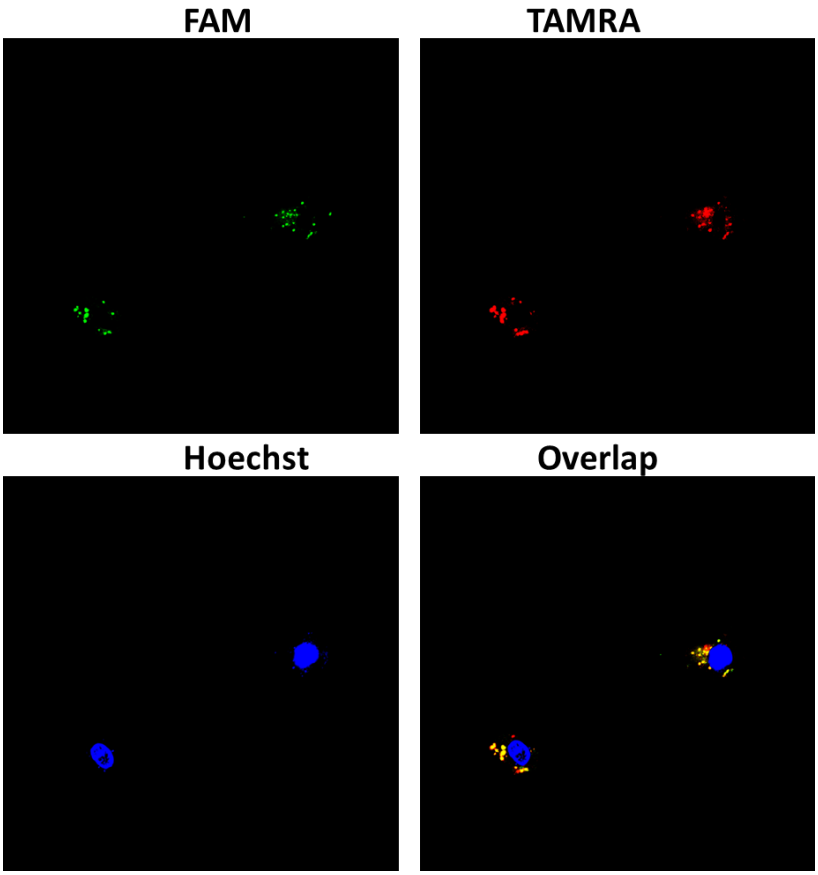


**Figure S8. Analyze of fluorescence colocalization.** The cell nucleus was stained with hoechst 33342. The merge image shows co-localization of both FAM and TAMRA in cytoplasm of Huh7 cells after incubated with various probes for 8 h. Green field = FAM fluorescence, and red field =TAMRA fluorescence. The FAM and TAMRA fluorescence emission channels were collected under an exictation laser at 488 nm. Excitation of hoechst 33342 was carried out at $\lambda$ = 405 nm and emissions was collected in the blue channel.

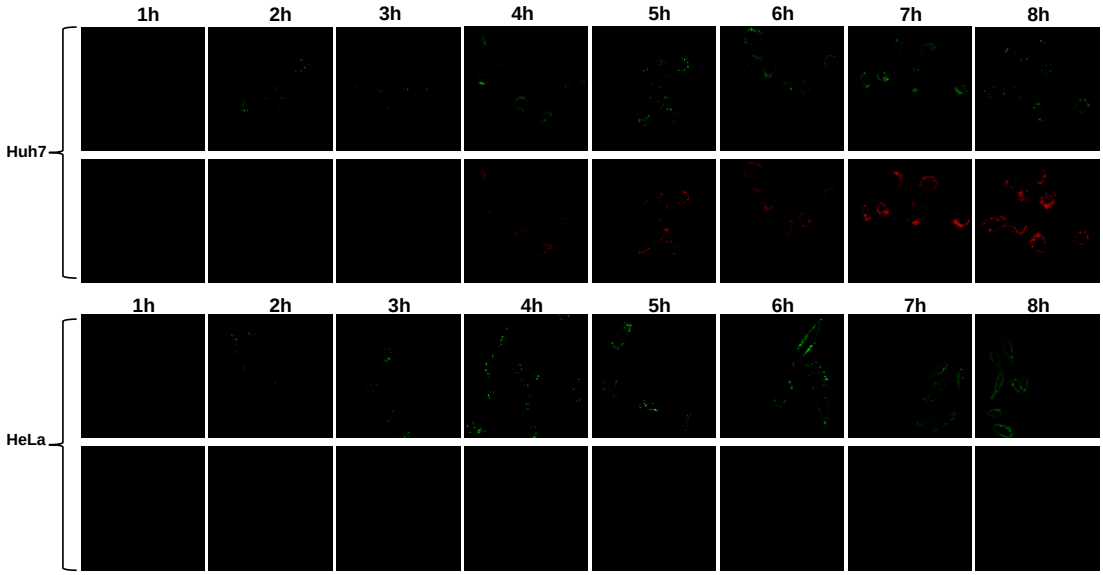


**Figure S9 Optimization of incubation time.** The Huh7 cells and control cells HeLa cells were treated with 100 nM (1)/200 nM (2) complex, 100 nM (4) and 200 nM (7) for 1, 2, 3, 4, 5, 6, 7 and 8 h at 37 $^{0}$C, respectively. Green field = FAM fluorescence, and red field =TAMRA fluorescence. The FAM and TAMRA fluorescence emission channels were collected under an excitation laser at 488 nm. The images were performed with 60 × oil immersion objective.

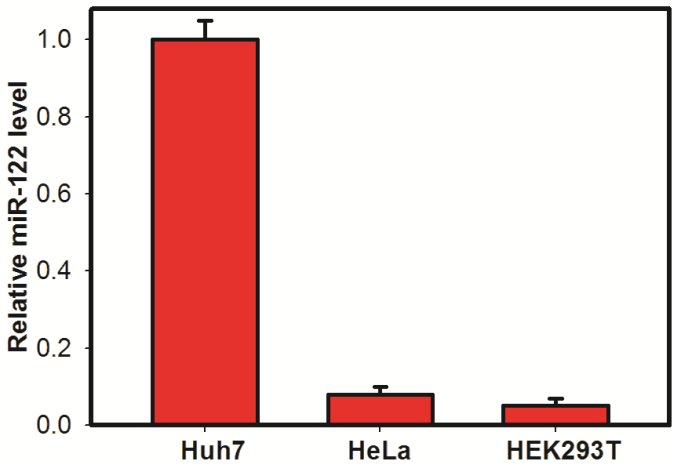


**Figure S10. Analyze of miR-122 in three different cells by qRT-PCR.** The histogram of the qPCR analysis represents the relative miR-21 level in different cells. The error bars were obtained from three independent measurements.

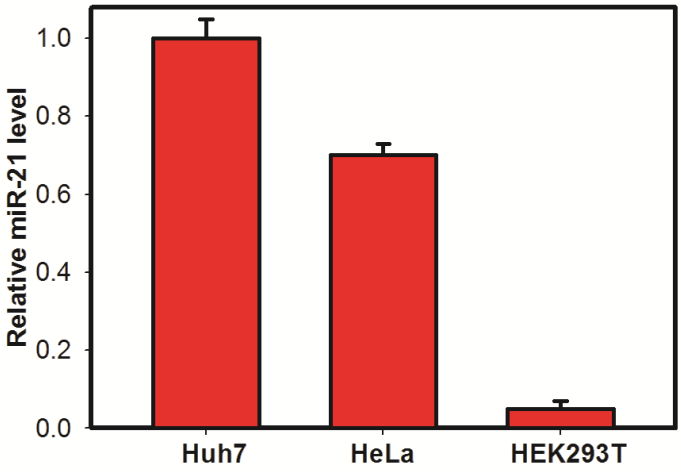


**Figure S11. Analyze of miR-21 in three different cells by qRT-PCR.** The histogram of the qPCR analysis represents the relative miR-21 level in different cells. The error bars were obtained from three independent measurements.

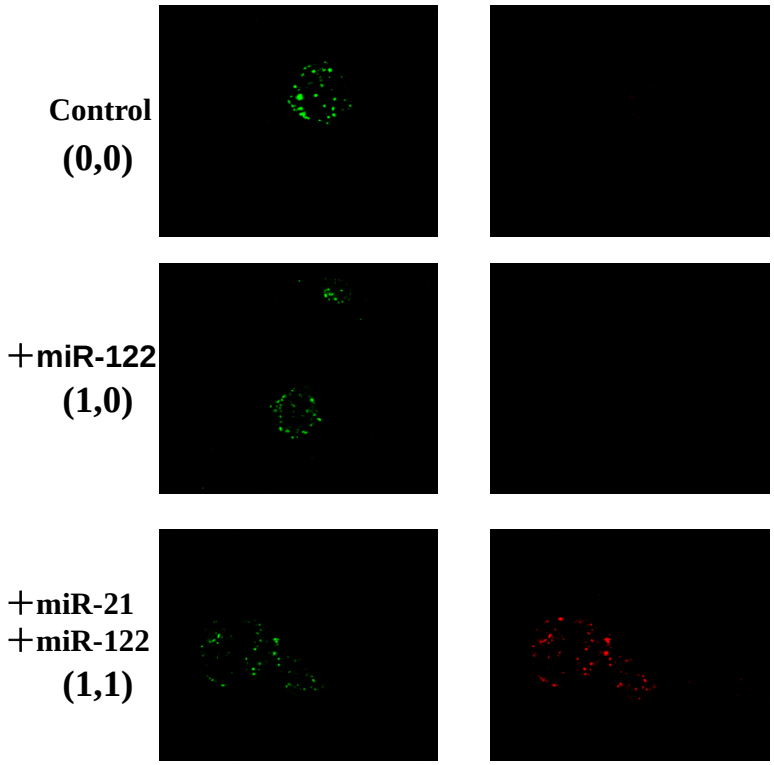


**Figure S12. Activation test of the cascade DNA circuits in HEK293T cells.** Confocal imaging of HEK293T cells for the verification of logic gate activation before (top) and after being transfected with 100 nM miR-122 (middle), 100 nM miR-122 and 100 nM miR-21 (bottom). Green field = FAM fluorescence, and red field =TAMRA fluorescence.

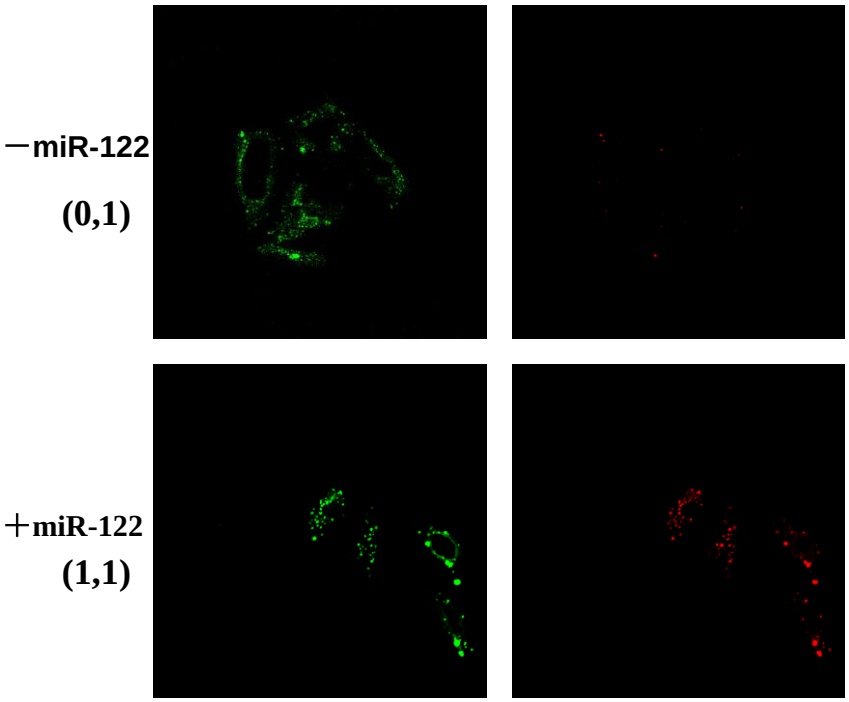


**Figure S13. Activation test of the cascade DNA circuits in HeLa cells.** Confocal imaging of HeLa cells for the verification of logic gate activation before (top) and after (bottom) being transfected with 100 nM miR-122. Green field = FAM fluorescence, and red field =TAMRA fluorescence.

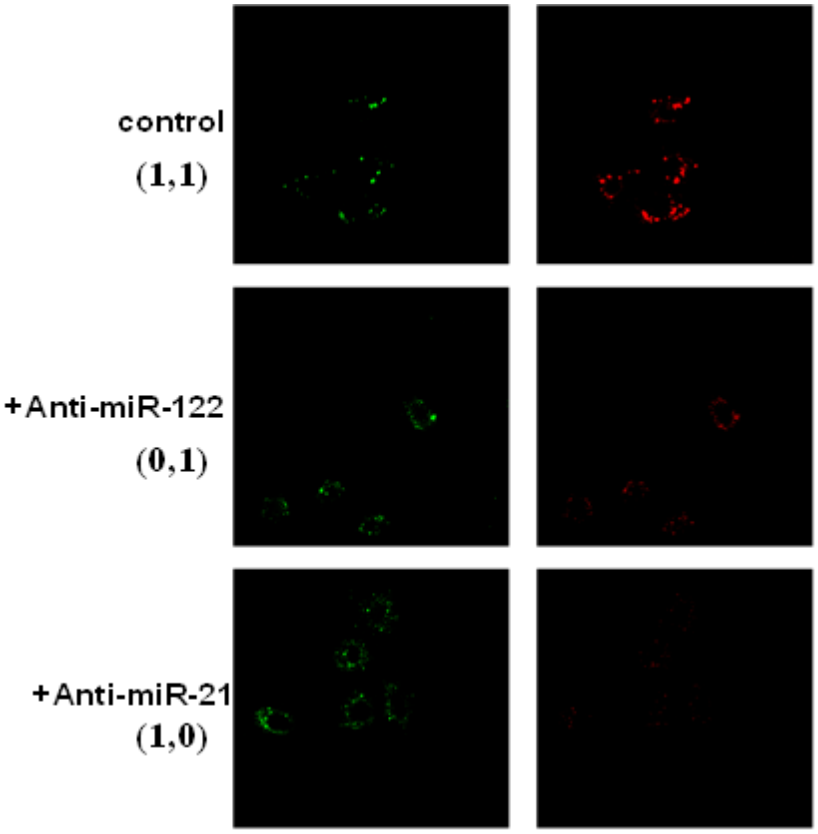


**Figure S14. Imaging of Huh7 cells before and after additional treatment with 200 nM anti-miR-21 or anti-miR-122 probe.** The sequences of anti-miR-21 and anti-miR-122 represent accurate complimentary copies of the miR-21 or miR-122 sequence. The imaging was performed with 60 × oil immersion objective.