Supporting Information

Aptamer-Array-Guided Protein Assembly Enhances Synthetic mRNA Switch Performance

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**SUPPORTING INFORMATION**

**Experimental Procedures**

**Cell cultures**

HEK293 and HepG2 cells were cultured in Dulbecco’s Modified Eagle’s Medium, high glucose (Gibco 11965) with 10% Fetal Bovine Serum (Sigma F2442) and 1% Non-essential amino acids (Sigma M7145). The HepG2-RFP cell line was prepared by transduction with ready-to-use RFP encoding lentivirus particles for the cell culture system (Amsbio LVP428) according to the manufacturer’s instructions. HepG2-RFP cells were sorted with BD FACS Aria III and selected by genetin selective antibiotic (G418 sulfate) before use. The Human iPS Cell 201B7 cell line was maintained on iMatrix-511 (Nippi.Inc) coating in complete StemFit medium (Ajinomoto AK02N). 201B7 cells were randomly differentiated through a 14-d culture in StemFit medium without basic fibroblast growth factors (FGFs) to obtain 201B7-d14 cells. The stemness of the 201B7 and 201B7-d14 cells were tested by the alkaline phosphatase staining kit (Sigma 86R) according to the manufacturer’s instructions followed by the EVOS XL Core Imaging System observation. All cells were cultured at 37 °C with 5% CO₂.

**dsDNA template generation for in vitro transcription**

All 5′-UTR sequences used in this project are listed in supporting information Table S1; all 3′-UTR sequences used in this project are listed in supporting information Table S2. Full MCP protein sequence was obtained from pCTp-MS2CP [4] and all MS2 RNA array DNA as well as pP7 RNA array DNA were synthesized by commercial company. PCP protein sequence was from plasmid pET22TevPP7CP(C68A,C73A) (addgene 27548), sfGFP N1-10 and N11 fragments were obtained from plasmid pCMV-N1-mouse-superfolderGFP (addgene 42143). Full mCaspase8 protein sequence was obtained from plasmid pBS-MmCasp8 (RIKEN, RD808071) and mCaspase8 was prepared by deletion of the first 177 residues of the N terminal (from Asp1 to Asp177). The linker sequence between MCP and the inactive monomers is ggaggaacct [5] the linker sequence between stGFPN11 and N1-10 is ggagaagattc [6] RNA fragment for EMSA was exact the MS2 RNA 12x51 array sequence with an addition of T7 promoter at 5′ end, iRFP670 full-length protein sequence was obtained from plasmid pUC19-iRFP670M9woT7r [5] DNA templates for all mRNAs and RNA fragments for in vitro transcription assay were generated by fusion PCR (Q5 HotStart HiFi polymerase, New England Biolabs) with attachment of a T7 promoter sequence. The concentration and the purity of templates was determined using the NanoVue Plus spectrophotometer (GE Healthcare).

**Synthesis and purification of mRNAs**

All mRNAs were synthesized by in vitro transcription (IVT) using MegaScript T7 Kit (Invitrogen). The mRNAs were synthesized with ATP, CTP, N°-methylpsuedouridine-5′-triphosphate and GTP and Anti Reverse Cap Analog (TriLink BioTechnologies), with a molar ratio at 5:5:5:1:4 [7] unless otherwise specified in the text. The reaction mixtures were incubated at 37 °C for 5 h and further incubated at 37 °C for 30 min in the presence of TURBO DNase (Invitrogen). RNA products were purified using the RNeasy MinElute Cleanup kit (QIAGEN) according to the manufacturer’s protocol and then treated with Antarctic Phosphatase (New England Biolabs) at 37 °C for 30 min. For the IRFP670 and PCP mRNA, a 120-nt polyA tails was encoded in the DNA template and was directly incorporated during IVT. For the other mRNAs, the polyA tails were added by treatment with E. coli Poly(A) Polymerase (New England Biolabs) at 37 °C for 36 min. The amounts and the purity of the product mRNAs were determined by NanoVue Plus Spectrophotometer (GE Healthcare) and Fragment Analyzer (Agilent Advanced Analytical Technologies Fragment Analyzer). The purified mRNAs were stored at -80 °C until use.

**Transfection**

For flow cytometry experiment, HEK293 cells were seeded into 48-well plates at 2.5×10⁴ cells/well in 0.25 mL/well of culture medium. For co-cultured flow cytometry experiments, HEK293 and HepG2-RFP cell line were seeded into 48-well plates in a ratio of 1:2 with 3.75×10⁴ cells/well in 0.25 mL/well of medium. For confocal experiment, HEK293 cells were seeded into confocal dishes at 1×10⁵ cells/well in 2 mL/well of culture medium. After 24 h, mRNAs were transfected using Lipofectamine MessengerMAX (Invitrogen) according to the manufacturer’s instructions. 201B7 and 201B7-d14 were seeded in 48-well plates with iMatrix-511 (Nippi.Inc) coating at a density of 2×10⁴ cells/well in 0.15 mL/well of appropriate medium with 10 μM ROCK inhibitor Y27632 respectively. After incubation for 24 h, media were replaced with the appropriate medium without Y27632, and mRNAs were transfected using Lipofectamine MessengerMAX (Invitrogen) according to the manufacturer’s instructions. The amounts of mRNA used in each experiment were listed in Table S3. For all fluorescence analysis measurements, all samples were co-transfected with 0.075 pmol iRFP670 mRNA; a sample transfected with only the IRFP670 mRNA was used as negative control.
Fluorescence analysis

24 h after transfection, the cells were trypsinized and suspended in culture media for flow cytometry analysis (Attune Nxt flow cytometer or BD FACs Aria III). The iRFP670 signal from the living cells was detected by excitation laser at 637 nm and emission filter at 670/14 nm on the Attune Nxt, or by excitation laser at 633 nm and emission filter at 660/20 nm on the BD FACS Aria III. The positively transfected cells, defined as the iRFP670 positive populations, were used in further analysis. GFP signals were detected using an excitation laser at 488 nm and emission filter at 530/30 nm. In the co-culture transfection experiments, RFP fluorescence was detected with an excitation laser at 561 nm and emission filter at 582/15 nm. The GFP mean fluorescent intensity in arbitrary unit were obtained from the machines directly. The arbitrary units of fluorescent intensity were calibrated using standardized fluorescent beads (Attune Performance Tracking Beads from Invitrogen or CS&T Research Beads from BD) according to the manufacturer’s instructions.

Confocal analysis

24 h after transfection, confocal dishes were directly applied to Leica SP8 Confocal Microscope. The imaging was made with 20× and 63× oil immersion objective using the 488 nm laser for excitation and the range of 500-600 nm for emission detection.

Viability analysis

24 h after transfection, cells were trypsinized and suspended in 250 μL of culture media. The cells were stained by adding 5 μL of 7-aflamoactinomycin D solution (eBioscience 7-AAD ready-to-use Viability Staining Solution, Invitrogen) to each sample followed by incubation at room temperature for 10 min. The samples were analyzed on the BD FACS aria III and 7-AAD signals were detected by excitation at 561 nm and emission at 670/14 nm. The living cells, defined as the 7-AAD-negative populations, were used for cell counting. The cell count of negative control sample, treated only with the transfection reagent, was set as full viability.

Expression of recombinant protein

MCP and MCP-Inverted sfGFP fusion protein ORFs were cloned into pET28a vector, and the recombinant proteins were expressed in Escherichia coli strain BL21 at 37 °C for 4 h with the addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 20 °C overnight with the addition of 0.25 mM IPTG, respectively. For MCP, cell pellets were collected by centrifugation and the recombinant proteins were purified using affinity chromatography column (Ni2+ resin, QIAGEN) under native condition according to manufacturer’s manual (QIAGEN). Buffer exchange was performed by dialysis twice in refolding buffer (50 mM Tris-Cl, 1 mM KCl, 2 mM MgCl2, pH 8.5).[6] For MCP-Inverted sfGFP fusion protein, lysis and purification were performed in denaturing buffer and then dialysed twice under the same refolding buffer. Samples were analyzed by SDS-PAGE and protein concentrations were determined by BCA assay (Pierce BCA Protein Assay Kit, Thermo Scientific).

EMSA assay

EMSA experiment protocol was based on previous literature.[2-7] In this assay, mRNA of MS2 aptamer array (51 nt Linker) was used. Generally, RNA samples were firstly annealed by incubation at 80 °C for 3 min and cooled at room temperature. In a total 20 μL mixture, 5×MCP binding buffer (100 mM HEPES-KOH, 750 mM KCl, 7.5 mM MgCl2, 10 mM DTT, pH 7.5), 50 nM RNA solution, 0.05 μL (2 U) RNase inhibitor (Promega RNasin plus Ribonuclease Inhibitor) and RNase-free water were mixed and incubated at room temperature for 10 min. 250-4000 nM proteins in 10×solution (400 mM Tris-Cl, 100 mM NaCl, 60 mM MgCl2, 100 mM DTT, 10 mM spermidine, pH 7.5) were then added to the mixture and incubated for 1 h at room temperature for MCP binding or 1 h at 37°C for MCP-Inverted sfGFP protein binding. For gel analysis, samples were applied to agarose gel (1.6% gel for MCP EMSA and 0.8% gel for MCP-Inverted sfGFP EMSA) for 25 min with constant voltage at 240 V in 0.5×TB (Tris-borate) buffer at 4 °C.[8] The gels were stained with Gel Red (Biotium) and the images were taken under UV (Bio-Rad Gel Documentation System).

Transmission electron microscopy

The protein/RNA complex sample was prepared as described in EMSA method. 10 μl of sample was deposited onto carbon-coated 400 mesh copper grids for 10 min and the excess liquid was blotted with filter paper. The sample was further washed twice by adding a drop of double distilled water for 2 sec and blotted each time. 2.5 μl of 2% (w/v) uranyl acetate was then added to the samples for 1 min and the excess liquid was blotted with filter paper.[9] The samples were left air-dried and applied to Field Emission Electron Microscope STEM (JEOL Model JEM-2100F).
Quantification and statistical analysis

Statistical values including the exact number of repeats (N = 3) and statistical significance were reported in the figure legends. Statistical analysis was performed using Microsoft Excel 2016 with the following formula: "Average ()" for mean values and "STDEV ()" for standard deviations. The significance levels were calculated through single-factor analysis of variance (ANOVA) by Microsoft Excel 2016 and denoted as *P < 0.05, **P < 0.01, ***P < 0.001. IC₅₀ values were calculated by nonlinear regression to fit a four-parameter logistic equation analysis using the Graphpad 8.0 software.
Supplementary Results and Discussion

As shown in Figure 1A, the Inverted sfGFP sample and the sfGFP sample emitted similar level of GFP signal, suggesting the free Inverted sfGFP proteins can randomly aggregate on their own, as demonstrated in previous works. By placing the MCP to the end of the Inverted sfGFP protein, such random aggregation can be successfully blocked, likely due to the steric hinderance introduced by the bulky MCP domain. The sfGFP-Array mRNA, carrying MCP sequence and a long aptamer array, is 2.2 times the length of the sfGFP mRNA. Regardless of this significantly difference in length, when the two mRNAs were transfected to cells at the same molar concentration, similar levels of cell viability and transfection efficiency on HEK293 cells were observed (Figure S1B), indicating that the two mRNAs induced similar transfection burden on the cells. That is, the longer design of the novel mRNA switch does not hinder the use of the mRNA switches. Importantly, similar differences in output performance were observed on the pair of mRNA switches when different concentrations of the switches were used (Figure S3B). These data together validated that the difference in output performance between the two mRNA switches is independent of their transfection condition.

In flow cytometry measurement of fluorophore signal from cells, both the fluorescent properties of the fluorophore itself and the effects of background fluorescence (such as autofluorescence from the cells) determine the resolution. This background fluorescence (system noise) induces system signal-to-noise ratio. It is calculated by dividing the median fluorescence intensity of the positive cells by that of the negative cells of each sample. As demonstrated in Figure S1C, the 12×51 array clearly exhibited stronger system signal-to-noise ratio, indicating a higher observation sensitivity. Importantly, the system signal-to-noise ratio of the MCP-Inverted sfGFP with 12×51 array mRNA is at a comparable level with that of the sfGFP mRNA, suggesting the MCP-Inverted sfGFP with 12×51 array mRNA exhibited sufficient resolution for fluorescence observation.

On all the output protein constructs, commonly used flexible linker sequences were placed between each domain/fragment. Such local flexibility among the domain favors intermolecular assembly of inactive protein domains of different sizes after the RBP domains bind onto the aptamer array.

To validate that the 12×51 aptamer array can trap MCP protein, we performed TEM experiment on 12×51 aptamer array RNA (858 nt) with MCP protein. We found that several dots of strongly stained proteins were observed along the RNA strand, indicating that MCP proteins were bound with the RNA. The heavy staining of the protein dots was likely the result of dye retention within the spaces between neighboring molecules.

In nature, upstream signaling induces protein scaffolding to enrich and drive the death effector domain (DED) on Caspase 8 protein to assemble, forming self-catalytic assemblies to induce downstream cell death. The strong cytotoxic effect from the Caspase 8-Array mRNA suggest that the aptamer array can mimic the natural upstream protein scaffolding to enrich and drive the self-catalytic assembly formation. By placing the MCP in the opposite terminus of the Caspase 8, the resulted Inverted Caspase 8 protein failed to respond to the aptamer array, suggesting the RBP should locate in the same position as the DED domain to allow proper Caspase 8 assembly (Figure S5).

At last, we explored whether other RBP-aptamer pairs can be used to build the readout control module. We constructed a miR-21-sfGFP-PP7-Array switch, carrying a PCP protein as the RBP domain of the output protein and a 12×51 PP7 aptamer array. The miR-21-sfGFP switch was, again, used as the reference switch. As shown in Figure S6, the miR-21-sfGFP-PP7-Array switch exhibited stronger output signal suppression by miR-21 mimic than the reference switch, suggesting this array switch also exhibit higher miRNA sensitivity. The histogram of the cells also clearly showed that the miR-21 mimic can suppress the GFP signal from miR-21-sfGFP-PP7-Array switch to the level close to the negative control, while the miR-21-sfGFP switch still express high level of GFP. These data validated that the readout control module can be built by different RBP-aptamer pairs.
Supplementary Figures and Tables

Figure S1. Performance of mRNA switch with inversely linked split protein as the inactive protein domain. (A) Protein expression comparison between sfGFP and sfGFP-Array mRNAs on HEK293 cells. (B) Relative cell viability and transfection efficiency on HEK293 cells transfected with sfGFP and sfGFP-Array mRNAs. (C) System signal-to-noise ratio showing the signal detection resolution of sfGFP and sfGFP-Array mRNAs on HEK293 cells. (D) Low magnification confocal images showing the subcellular distribution of fluorescent signals generated from sfGFP mRNA and sfGFP-Array mRNAs. N = 3; data are presented as the mean ± SD.

Figure S2. Electrophoretic mobility shift and TEM images of the aptamer array with proteins. (A) Electrophoretic mobility shift of the 12×51 aptamer array RNA (858 nt; 278.5 kDa) with recombinant MCP and recombinant MCP-Inverted sfGFP proteins. The band positions of 1016 nt reference RNA (*), 12×51 aptamer array RNA (†), and the protein-RNA complexes (‡) are indicated on the left of the gels. The broad smears continuously retard with increasing concentrations of both MCP and MCP-Inverted sfGFP proteins, indicating increasingly protein-bound RNAs. (B) Representative TEM image showing 12×51 aptamer array RNA with recombinant MCP protein. The black arrows indicate the RNA strand; the white arrows indicate the dots of proteins bound onto the RNA.
Figure S3. The readout control module enhances the performance of model mRNA switches. (A) The output GFP signals from the two switches without or with miR-21 mimic. The GFP signals from cells transfected with only the reference iRFP670 mRNAs were used as negative control. (B) The miRNA sensitivity of the pair of miR-21-sensing switches. The switches were used at high concentration (0.6 pmol). N = 3; data are presented as the mean ± SD.

Figure S4. Representative dot-plot images showing the comparison of output GFP intensity among the miR-21-positive cell populations in the co-culture after transfection with the miR-21-sensing switches or just the reference iRFP670 mRNAs (negative control). N = 3; data are presented as the mean ± SD.
Figure S5. Engineered mRNA switch with assembly-deficient proteins have higher performance efficiency. (A) Scheme of engineered protein with MCP fused to the opposite end of dCaspase 8. Cytotoxicity of mRNAs encoding different engineered proteins. (B) Representative images showing the 201B7 and 201B7-d14 cells. The stemness of the cells were demonstrated by the red-violet alkaline phosphatase staining. (C) Cell-specific elimination performance by the pair of switches with synthetic cap analogs: m32.2.7GP3G (3Met) cap and 3’-O-Me-m7G(5’)-ppp(5’)-G (ARCA) cap. N = 3; data are presented as the mean ± SD. **P < 0.01 calculated through single-factor analysis of variance (ANOVA).

Figure S6. Performance of mRNA switch with readout control module built on PCP-PP7 pair. The GFP positive cell populations of the pair of miR-21-sensing switches on HEK 293 cells. N = 3; data are presented as the mean ± SD. Representative histogram plots showing the change of GFP signals. The histograms of the test samples were shown in red; the histogram of HEK293 cells without mRNA transfection was shown in blue as the negative control. *P < 0.05, **P < 0.01, ***P < 0.001 calculated through single-factor analysis of variance (ANOVA).
### Table S1. 5′-UTR sequences used on the mRNAs in this project.

| Name         | Sequence (5′-3′)                                                                 | mRNAs containing this UTR sequence                                                                 | References |
|--------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|------------|
| 5′-UTR       | GGCGGAGAUAGAAGAGAAAGGAGAUAAGAAGAAUAAGAGCAGCGGGUGCCCAACUAG                      | stGFP, Inverted stGFP, MCP-Inverted stGFP, MCP-Inverted stGFP-Array (10 Linker), MCP- Inverted stGFP-Array (20nt Linker), stGFP-Array, Caspase 8, dCaspase 8, MCP-dCaspase 8, Caspase 8-Array, dCaspase 8-Array, MRC-PP70, PCP | [5]        |
| 5′-UTR       | GGCGCAUCUCACAGUCGUCAGGCGUAGAAGCCCACGCAACCAAGAUAGAAGAAAGGAGAUAAGAAGAAUAAGAGCAGCGGGUGCCCAACUAG   | miR-21-stGFP Switch, miR-21-stGFP-Array Switch, miR21-stGFP-PP7-Array Switch                  | [5]        |
| 3′+PP7-5′    | GGCGCAGUGUGUAGAAGAAAGGAGAUAAGAAGAAUAAGAGCAGCGGGUGCCCAACUAG                      | PCP-stGFP Switch, PCP-stGFP-Array Switch                                                          | [5]        |
| 3′-UTR       | GGCGCAGUGUGUAGAAGAAAGGAGAUAAGAAGAAUAAGAGCAGCGGGUGCCCAACUAG                      | miR-302-Caspase 8 Switch, miR-302-Caspase 8-Array Switch                                         | [5]        |

The underlined letters indicate the sequences of PP7 aptamer or reverse complementary sequences of miRNA. The lowercase letters indicate linker sequences.

### Table S2. 3′-UTR sequences used on the mRNAs in this project.

| Name           | Sequence (5′-3′)                                                                 | mRNA containing this UTR sequence                                                                 | References |
|----------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|------------|
| 3′-UTR         | UAGUCUAGAGCUUCUCGCUGGCGCGGUGCUCUGCGCCAUAGCUAGAAGAGAUAAGAAGAAUAAGAGCAGCGGGUGCCCAACUAG | stGFP, Inverted stGFP, MCP-Inverted stGFP, miR-21-stGFP Switch, Caspase 8, dCaspase 8, MCP-dCaspase 8, miR302-Caspase 8 Switch, MRC-PP70, PCP | [5]        |
| 12+10 Array-3′-UTR | UAAACCAUACACACGACUCUUGCAUUGAAGAAGAACCAUUGAAGAAGAAUAAGAGCAGCGGGUGCCCAACUAG | MCP-Inverted stGFP (10 Linker)                                                                 | [11]       |
| 12-20 Array-3′-UTR | UAAACCAUACACACGACUCUUGCAUUGAAGAAGAACCAUUGAAGAAGAAUAAGAGCAGCGGGUGCCCAACUAG | MCP-Inverted stGFP (10 Linker)                                                                 | [11]       |
| 12+51 Array-3′-UTR | UAAACCAUACACACGACUCUUGCAUUGAAGAAGAACCAUUGAAGAAGAAUAAGAGCAGCGGGUGCCCAACUAG | miR-21-stGFP Switch, dCaspase 8-Array, Caspase 8-Array, MRC-PP70, PCP | [5]        |

RNAi indicates linker sequences.

References:

[1] ... [13]...
The underlined letters indicate the sequence of MS2 aptamer array or PP7 aptamer array. The lowercase letters indicate linker sequences.

| Transfection Experiment | Amount of test mRNAs (pmol) | Amount of internal control mRNA (pmol) | Culture condition |
|-------------------------|-----------------------------|---------------------------------------|-------------------|
| Figure 1A               | sfGFP (0.6 pmol)            | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | Inverted sfGFP (0.6 pmol)  | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | MCP-Inverted sfGFP (0.6 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
| Figure 1C               | sfGFP (0.6 pmol)            | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | sfGFP-Array (0.6 pmol)      | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
| Figure 1D, S1D          | sfGFP (2.4 pmol)            | N/A                                   | Confocal dist/2 mL  |
|                         | sfGFP-Array (2.4 pmol)      | N/A                                   | Confocal dist/2 mL  |
| Figure 2B, S3A          | miR-21-sfGFP Switch (0.15 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | miR-21-sfGFP-Array Switch (0.15 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
| Figure 2C, S4           | sfGFP (0.6 pmol)            | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | sfGFP-Array (0.6 pmol)      | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | miR-21-sfGFP Switch (0.6 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | miR-21-sfGFP-Array Switch (0.6 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
| Figure 2D               | PCP-sfGFP Switch (0.15 pmol), PCP (0 pmol, 0.3 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | PCP-sfGFP-Array Switch (0.15 pmol), PCP (0 pmol, 0.3 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
| Figure 3C, S5C          | miR-302-Caspase 8 Switch (0.05 pmol) | N/A                                   | 48-well plate/0.15 mL |
|                         | miR-302-Caspase 8-Array Switch (0.05 pmol) | N/A                                   | 48-well plate/0.15 mL |
| Figure S1A, S1B         | sfGFP (0.6 pmol)            | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | sfGFP-Array (0.6 pmol)      | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
| Figure S1C              | sfGFP (0.6 pmol)            | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | MCP-Inverted sfGFP-Array (10nt Linker) (0.6 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | MCP-Inverted sfGFP-Array (20nt Linker) (0.6 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | sfGFP (0.6 pmol)            | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
| Figure S3B              | miR-21-sfGFP Switch (0.6 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | miR-21-sfGFP-Array Switch (0.6 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
| Figure S6               | miR-21-sfGFP Switch (0.15 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
|                         | miR-21-sfGFP-PP7-Array Switch (0.15 pmol) | iRFP670 (0.075 pmol)                  | 48-well plate/0.25 mL |
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Author Contributions

Y.K. conceived the project. Y.K. and Q.L. designed the experiments. Q.L. performed most of the experiments and analyzed the experiment data. Y.H. and C.Y.L. performed the validation experiments. Y.H. verified the data processing and analysis. Y.K. and Q.L. wrote the manuscript.