

Synthetic circular RNA switches and circuits that control protein expression in mammalian cells

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ABSTRACT

Synthetic messenger RNA (mRNA) has been focused on as an emerging application for mRNA-based therapies and vaccinations. Recently, synthetic circular RNAs (circRNAs) have shown promise as a new class of synthetic mRNA that enables superior stability and persistent gene expression in cells. However, translational control of circRNA remained challenging. Here, we develop ‘circRNA switches’ capable of controlling protein expression from circRNA by sensing intracellular RNA or proteins. We designed microRNA (miRNA) and protein-responsive circRNA switches by inserting miRNA-binding or protein-binding sequences into untranslated regions (UTRs), or Coxsackievirus B3 Internal Ribosome Entry Site (CVB3 IRES), respectively. Engineered circRNAs efficiently expressed reporter proteins without inducing severe cell cytotoxicity and immunogenicity, and responded to target miRNAs or proteins, controlling translation levels from circRNA in a cell type-specific manner. Moreover, we constructed circRNA-based gene circuits that selectively activated translation by detecting endogenous miRNA, by connecting miRNA and protein-responsive circRNAs. The designed circRNA circuits performed better than the linear mRNA-based circuits in terms of persistent expression levels. Synthetic circRNA devices provide new insights into RNA engineering and have a potential for RNA synthetic biology and therapies.

INTRODUCTION

Gene delivery using synthetic messenger RNA (mRNA) is an effective method for transient gene expression, with a reduced risk of genomic integration in the cell (1). One of the limitations hindering its broader application for medical research is lower gene expression persistence caused by its instability. To improve the stability and performance of

synthetic mRNA, substantial efforts have been made to engineer new RNA structures and, in recent years, synthetic circular RNAs (circRNAs) have shown promise as a new class of synthetic mRNA with superior stability and persistent gene expression (2–4).

Endogenous circRNAs are generated through back-splicing (5). When first discovered, they were thought to be products of splicing errors (6). However, they have since been reported to be involved in various biological functions as either protein-coding or non-coding RNA (7). Additionally, these circRNAs resist exonuclease-mediated degradation as their covalent-closed loop structure lacks free 5' and 3' ends. This common structural feature allows circRNAs to exhibit a longer half-life than linear mRNAs in cells (7,8).

To expand the potential of mRNA therapeutics, it is important to produce desired outputs depending on the cell state and reduce off-target effects in non-target cells and tissues (9,10). Such ‘smart mRNA’ could be a useful tool for cell type-specific gene regulation and future therapeutic applications (11,12). Even before the function of endogenous circRNAs was determined, there had been efforts to adapt the circular structure to improve the stability of synthetic RNAs (13,14). However, research in the field of circRNA is relatively early, and studies have mainly focused on the methods of cyclization (3), the immunogenicity (15–18), and the performance of translation (3,19). Design principles of circRNAs that sense intracellular conditions and autonomously control their translation level remain unknown. Defining these principles would be useful in the development of therapies with suppressed off-target expression in non-target cells, maximizing the therapeutic effect.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate protein expression from mRNAs via translational repression and mRNA degradation (20). As the activity of miRNAs differs among cell types, they can be used as an indicator to distinguish between various cell types (21). We have previously developed miRNA-responsive linear mRNA (miRNA-responsive switch), which is composed of a protein-coding sequence and an antisense sequence to the target of the miRNA (anti-miR) at the untranslated region (UTR), enabling the distinguishment of cell types based on

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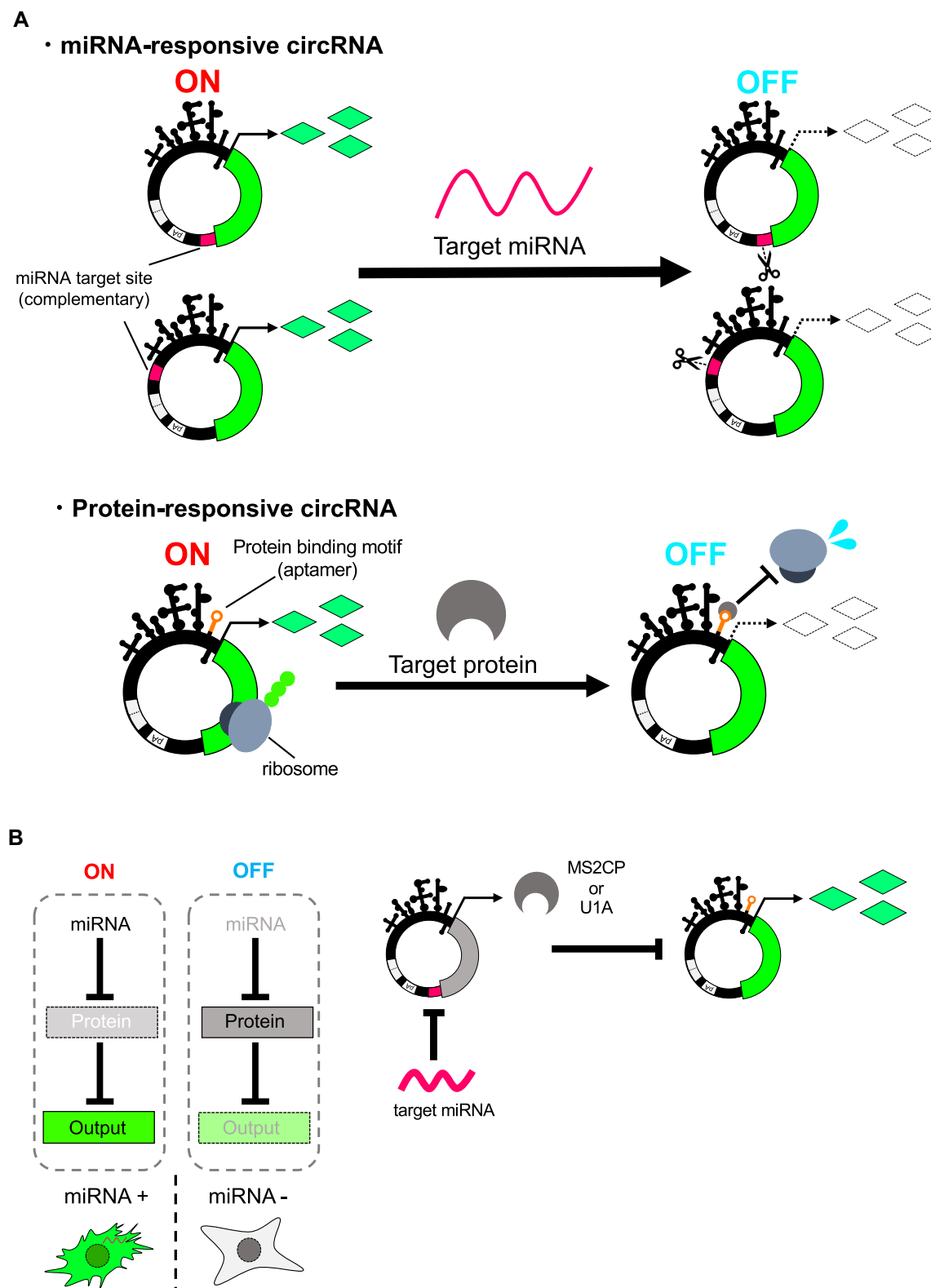


Figure 1. Schematic illustrations of circRNA switches and circuits. (A) Design of miRNA or protein-responsive circRNA switch. miRNA-responsive circRNA has the antisense of target miRNA sequence at the UTR. Protein-responsive circRNA has a protein-binding motif in IRES region. In both systems, gene expression from circRNA is repressed if the target miRNA or protein is present. (B) Scheme of circRNA circuit composed of miRNA- and protein-responsive circRNA switches. The first output (MS2CP or U1A protein) is encoded on a miRNA-responsive circRNA switch, and the second output (reporter protein) is encoded on a protein-responsive circRNA switch. In the OFF state (absence of input miRNAs), MS2CP or U1A protein represses translation of the second output gene-coding circRNA. In ON state (presence of input miRNAs), the MS2CP or U1A translation is repressed by the miRNAs, which leads to output translation.

sodium acetate pH 5.2, 0.1% SDS). The eluted RNAs were purified by phenol-chloroform extraction and precipitated with isopropanol. After dissolving the RNA pellet in nuclease-free water, RNAs were desalted using Amicon Ultra 0.5 ml Centrifugal Filters Ultracel-50K (Millipore), then incubated with Antarctic Phosphatase (New England Biolabs) at 37°C for 30 min. Phosphatase-treated RNAs were re-purified by phenol-chloroform extraction and isopropanol-precipitation. In this study, all circRNAs and linear mRNAs were purified from polyacrylamide gel, except for linear mRNAs used in Figure 5C to screen the appropriate variant. Concentrations of purified RNAs were measured by NanoDrop2000 (Thermo Fisher Scientific) and used in cellular experiments. All RNA sequences used in this research are described in Supplementary Sequences.

shRNA preparation for U1A knockdown

For transcribing shRNAs, a single-strand DNA templates (U1A-shRNA 5'-CTGATCAAGAAGGATGAGCTAA AAAAGCTATGCTCTTTTGTAGCTCATCCTTCTTG ATCTATAGTGAGTCGTATTAGC-3', Control-shRNA 5'-CTGCCTAAGGTTAAGTCGCCCTCGCCTATGC TGCAGGGGCGACTTAACCTTAGGCTATAGTGA GTCGTATTAGC-3') were annealed to T7 forward primer (5'-GCTAATACGACTCACTATAG-3'). Generated partial double-stranded templates were transcribed by using MEGAshortscript T7 Kit (Thermo Fisher Scientific). IVT reaction mixtures were incubated at 37°C for 16 h and then mixed with TURBO DNase (Thermo Fisher Scientific), and additionally incubated at 37°C for 30 min to remove the template DNA. After IVT and template removal, shRNAs were purified by the same procedures described in 'Synthesis and purification of circRNA and linear mRNA' with 12% denaturing PAGE and isopropanol-precipitation using Gene-Packman Coprecipitant (Nacalai Tesque).

The shRNA sequences (30) were as follows:

U1A-shRNA 5'-GAUCAAGAAGGAUGAGCUAAA AAAGAGCAUAGCUUUUUUAGCUCAUCCUUC UUGAUCAG-3'

Control-shRNA 5'-GCCUAAGGUUAAGUCGCCCCU CGCAGCAUAGGCGAGGGGCGACUUAACCUUA GGCAG-3'

Cell culture and RNA transfection

HEK293FT (Invitrogen), HeLa CCL2 (ATCC) and A549 (RCB3677) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) 4.5 g/l glucose (Nacalai Tesque) supplemented with 10% fetal bovine serum (FBS) (Biocera, Ireland Origin), 0.1 mM MEM non-essential amino acids (Life Technologies), 2 mM L-glutamine (Life Technologies) and 1 mM sodium pyruvate (Nacalai Tesque). All cell lines were cultured at 37°C with 5% CO₂. All transfections were performed using Lipofectamine MessengerMAX (Thermo Fisher Scientific) according to the manufacturer's protocol. RNAs were co-transfected with synthetic miRNA mimics or inhibitors (Thermo Fisher Scientific) in the case of miRNA-responsive switch experiments. In the case of the RBP-responsive switch experiments, the target RBP was ex-

pressed in cells by co-transfecting with RBP-coding mRNAs. The transfection condition details of each experiment are shown in Supplementary Table S1.

RNase R digestion assay

2.5 µg of *in vitro*-transcribed RNAs (Circular EGFP ΔpAΔIRES) were incubated at 37°C with 10 U of RNase R (Cosmo Bio) in a 10 µl mixture. After a 45 min incubation, the mixture was subjected to 4 and 8% denaturing PAGE (8.3 M urea), and then stained by SYBR Green II Nucleic Acid Gel Stain (TaKaRa). Stained RNA was detected by Typhoon FLA-7000 (GE Healthcare).

Splice junction sequencing

Circular EGFP ΔpAΔIRES purified from denaturing polyacrylamide gel was reverse transcribed using reverse transcription primer (5'-CCTACTCAGGCTTTATTCA AAGACCAAG-3') and SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific). Reverse-transcribed cDNA was used as a template for PCR using PrimeSTAR Max DNA Polymerase (TaKaRa) with primer set for splice junction amplification (Fwd: 5'-agctcgccgaccactaccagcag -3', Rev: 5'-gtagcgctgaagcactgcagc-3'). Amplified product was purified by Monarch DNA Gel Extraction Kit (New England Biolabs), and then sequenced by the same method described in 'Template plasmid construction for mRNA'.

Flow cytometry and data analysis

HEK293FT, A549 (1.0×10^5 cells), and HeLa (0.5×10^5 cells) cells were seeded onto 24-well plates 24 h before transfection. All flow cytometry measurements were performed 24 h after the transfection using BD Accuri C6 (BD Biosciences). Cells were washed with phosphate buffered saline (PBS, Nacalai Tesque), trypsinized with 100 µl of 0.25% Trypsin-EDTA (Thermo Fisher Scientific), and incubated at 37°C for 5 min. After incubation, 150 µl of fresh medium was added. Cells were transferred to a fresh microcentrifuge tube passing through a nylon mesh. EGFP was detected by FL1 (533/30 nm, 99% attenuated), and iRFP670 was detected by FL4 (675/25 nm) filters, respectively. Collected data were analyzed using FlowJo 10.5.3 software. For data analysis, gates were generated by using mock samples. Data from the debris were removed when preparing forward versus side dot plots (FSC-A versus SSC-A). Then, events on the chart edges in the dot plots of FL-1 versus FL-4 were removed. In the histogram where iRFP670-intensity is displayed on the X-axis, the iRFP670-positive (reference-positive) gate was defined by a mock sample with 99.9% cells outside the gate. In the following analysis, the mean of EGFP+/iRFP670+ was used for calculation.

RT-qPCR analysis

For the RT-qPCR targeting immune response-related genes, A549 (1.0×10^5 cells) cells were seeded onto 24-well plates 24 h before transfection. Then, cells were washed with 1 ml of PBS and total RNA was extracted 24 h after transfection. To induce immune response-related genes, 200 ng of

Polyinosinic-polycytidylic acid [Poly(I:C)] (Enzo Life Sciences, Inc.) was transfected as a positive control. Total RNA extraction was performed using TRIzol Reagent (Thermo Fisher Scientific) and Monarch RNA Cleanup Kit (New England Biolabs) according to the manufacturer's protocol. 400 ng total RNA was used as a template for reverse transcription performed using ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO) in a 10 μ l reaction mixture. All optional steps described in the kit manual were applied.

For the RT-qPCR targeting synthetic linear mRNAs and circRNAs (Supplementary Figures S2B and S4B), HEK293FT (1.0×10^5 cells) cells were seeded onto 24-well plates 24 h before transfection. Then, total RNA was extracted at each time point after transfection. Extracted total RNA was treated with TURBO DNase (Thermo Fisher Scientific) and re-purified by Monarch RNA Cleanup Kit (New England Biolabs) according to the manufacturer's protocol. Reverse transcription was performed using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) with 100 ng total RNA template and 20 μ l reaction volume. In both cases, the synthesized cDNA solution was diluted by nuclease-free water with 5-fold dilution. One μ l of the diluted-cDNA solution was analyzed by qPCR. The qPCR analysis was performed using THUNDERBIRD Next SYBR qPCR Mix (TOYOBO) with 20 μ l reaction mixture and QuantStudio 3 Real-time PCR Systems (Thermo Fisher Scientific) following the manufacturer's protocol, with three steps reaction. Target mRNA quantities were normalized by ATP5B mRNA. All qPCRs were performed in technical duplicates and the averages of Ct were processed to calculate relative expression levels using the Δ Ct or $\Delta\Delta$ Ct method. The primers for qPCR are listed in Supplementary Table S2.

Western blot analysis

HEK293FT (1.0×10^5 cells) cells were seeded onto 24-well plates 24 h before transfection. 24 h after transfection, cells were washed with 1 ml of PBS and lysed in 50 μ l of RIPA buffer (Nacalai Tesque). The cell lysates were analyzed with subsequent western blotting as previously performed (30). The transferred membranes were incubated with specific primary antibodies, Anti-SNRPA (Santa Cruz Biotechnology, 200-fold dilution) and Anti-Enterobacterio Phage MS2 Coat Protein (Sigma-Aldrich, 5000-fold dilution), respectively. Anti-GAPDH antibody (Santa Cruz Biotechnology) was used at 500-fold dilution. Then, the blot was incubated with secondary antibodies. Goat Anti-Mouse IgG (H + L)-HRP conjugate (Bio-Rad) or Goat anti-Rabbit IgG (H + L)-HRP conjugate (BIO-RAD) was used at 400-fold dilution. All incubation steps were performed using iBind Flex Western Device (Thermo Fisher Scientific). Detection of the blot was performed with ECL Prime Western Blotting Detection Reagent (GE Healthcare) and Amersham ImageQuant 800 (Cytiva). The protein expression level was calculated from band intensities with ImageJ (NIH).

WST-1 assay

HEK293FT, A549 (2.0×10^4 cells) and HeLa (1.0×10^4 cells) were seeded onto 96-well plates 24 h before transfection.

24 h after transfection, 10 μ l/well of WST-1 reagent (Sigma-Aldrich) was added to the medium of each well, and the plates were incubated for 1 h at 37°C. After the incubation, the absorbance of 440 and 620 nm was measured by PE Envision 2104 Multilabel Reader (PerkinElmer).

Secreted luciferase assay

HEK293FT, A549 (1.5×10^4 cells) and HeLa (0.5×10^4 cells) cells were seeded onto 24-well plates 24 h before transfection. After transfection, culture media was harvested and replaced every 24 h up to 5 days (120 h) at each time point. Culture media was harvested and replaced for 24–120 h after transfection at each time point. Media was harvested into Protein LoBind tubes (Eppendorf) and stored at -30°C . To detect bioluminescence from Metridia Luciferase (MetLuc2), 50 μ l of harvested media was transferred into a Greiner LUMITRA 200 microplate (Greiner), and then 10 μ l of $0.5\times$ substrate/reaction buffer from Ready-To-Glow Secreted Luciferase Reporter Assay (TaKaRa) was added by injector attached on a plate reader. After 30s double orbit shaking with 3.0 mm diameter and 30s incubation delay, luminescence was detected by Centro LB 960 (Berthold Technologies) with an integration time of 1 s. Normalized MetLuc activity was calculated by normalizing the MetLuc activity 24 h after transfection.

Statistical analysis

Statistical values including the exact n and statistical test are reported in the figure legends. The levels of significance are denoted as $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. N.S. means non-significant ($0.05 < P$). All statistical tests were performed by Dunnett's test, and two-tailed unpaired Student's or Welch's *t*-test using R or Excel (Microsoft). The type of *t*-test was determined by *F*-test.

RESULTS

Design and evaluation of synthetic circRNAs

Several strategies for *in vitro* mRNA cyclization have been reported (39). We chose an engineered Permuted Intron-Exon (PIE) Splicing system, which is an efficient circularization method with no enzymatic treatment for long circRNA construction (3). We designed a circRNA construct that had permuted split fragments of group I catalytic introns corresponding to *Anabaena* pre-tRNA with homology arms at both ends, and a CVB3 IRES upstream of the open reading frame (ORF) (Figure 2A). The circRNA construct with splice sites resulted in a migrated band expected as a circular RNA in denaturing PAGE (Figure 2B), which was confirmed to be circRNA through splice junction identification with ligated 5' and 3' splice sites (Figure 2C). Resistance to RNase R was confirmed by observing a band of the migrated product of circRNA even after RNase R treatment whereas other linear products were degraded by the treatment (Figure 2D). RNA purification from polyacrylamide gels removed most of the impurities as reported previously (3,16) (Supplementary Figure S1). These data indicate that our construct generated circRNA products as

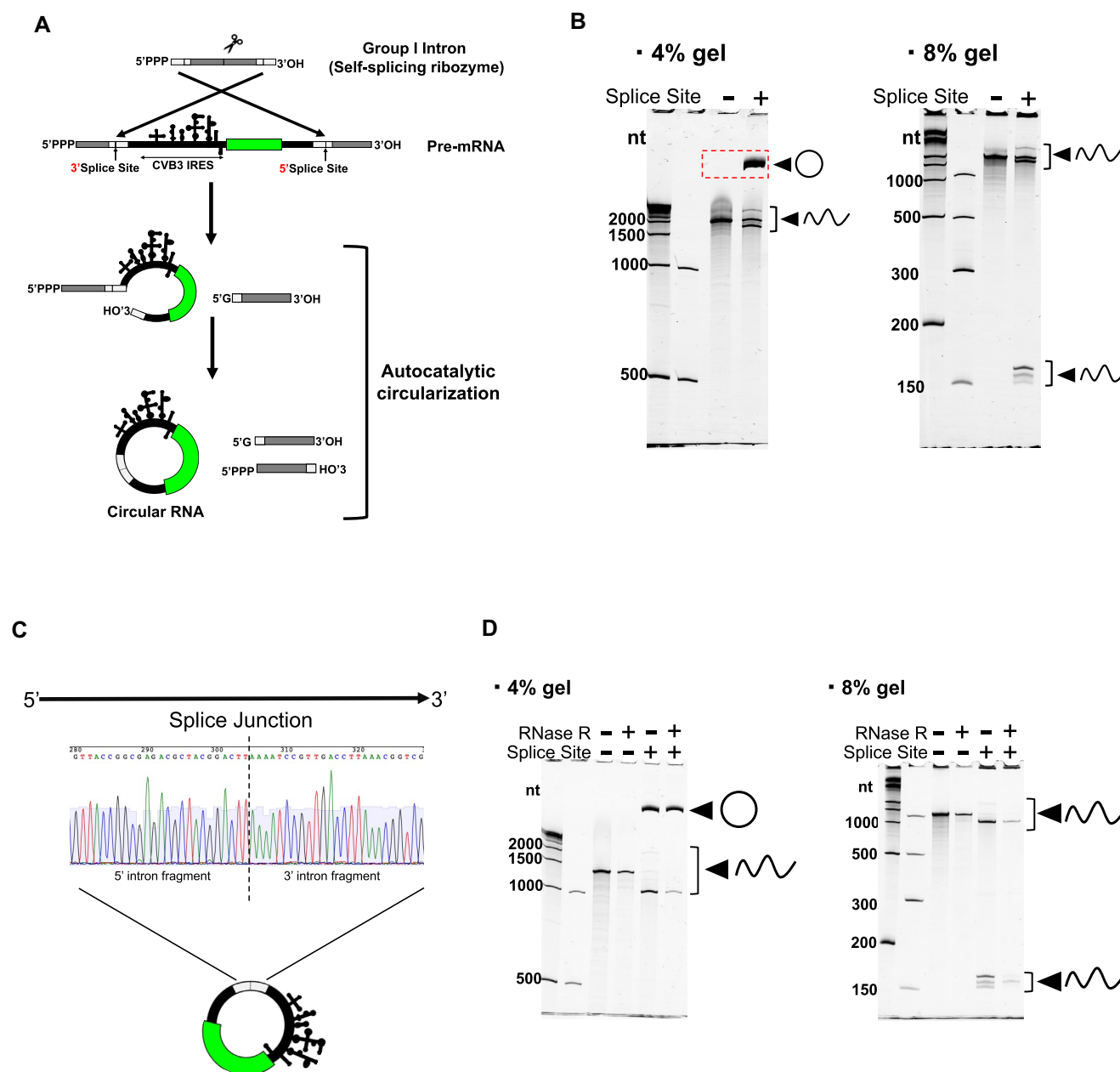


Figure 2. circRNA construction by permuted intron-exon (PIE) splicing. (A) Schematic illustration of PIE splicing. (B) Denaturing PAGE gel images after *in vitro* transcribed circRNA (Δ pA). DynaMarker RNA High (BioDynamics Laboratory) and Low Range ssRNA Ladder (NEB) were used as molecular weight markers. In the 4% gel, 4 bands (Splice Site +) represented as linear products are predicted to be 3805, 2060, 1910 and 1745 nucleotides (nt) in length from top to bottom. Linear product (Splice Site -) is predicted to be 2041 nt. In the 8% gel, bands at the bottom (Splice Site +) are predicted to contain linear products after splicing (the predicted length is 164 and 151 nt). (C) Sanger sequencing result of RT-PCR amplifying splice junction. (D) Denaturing PAGE gel images after RNase R treatment. In the 4% gel, four bands (RNase R -, Splice Site +) represented as linear products are predicted to be 2323, 1319, 1168 and 1004 nt in length from top to bottom. Linear product (Splice Site -) is predicted to be 1300 nt. Gel imaging experiments were repeated independently, at least twice.

expected. Previous studies reported that such features are unique in PIE Splicing-derived circRNA (3,18).

Next, we examined the translatability of synthesized circRNAs and the effect of circularization. We compared the levels of protein production from circRNAs with those of linear mRNAs (linRNAs). Overall protein expression levels from circRNAs and linRNAs were evaluated using re-

porter expression (EGFP) normalized by transfection control (iRFP670). We prepared different types of RNA, including four types of circRNAs with or without 120 nucleotides of polyA (pA) sequences (circRNA + pA, circRNA Δ pA), without IRES (circRNA + pA Δ IRES, circRNA Δ pA Δ IRES), and three types of linRNAs (Linear EGFP, Cap-EGFP) (Figure 3A).

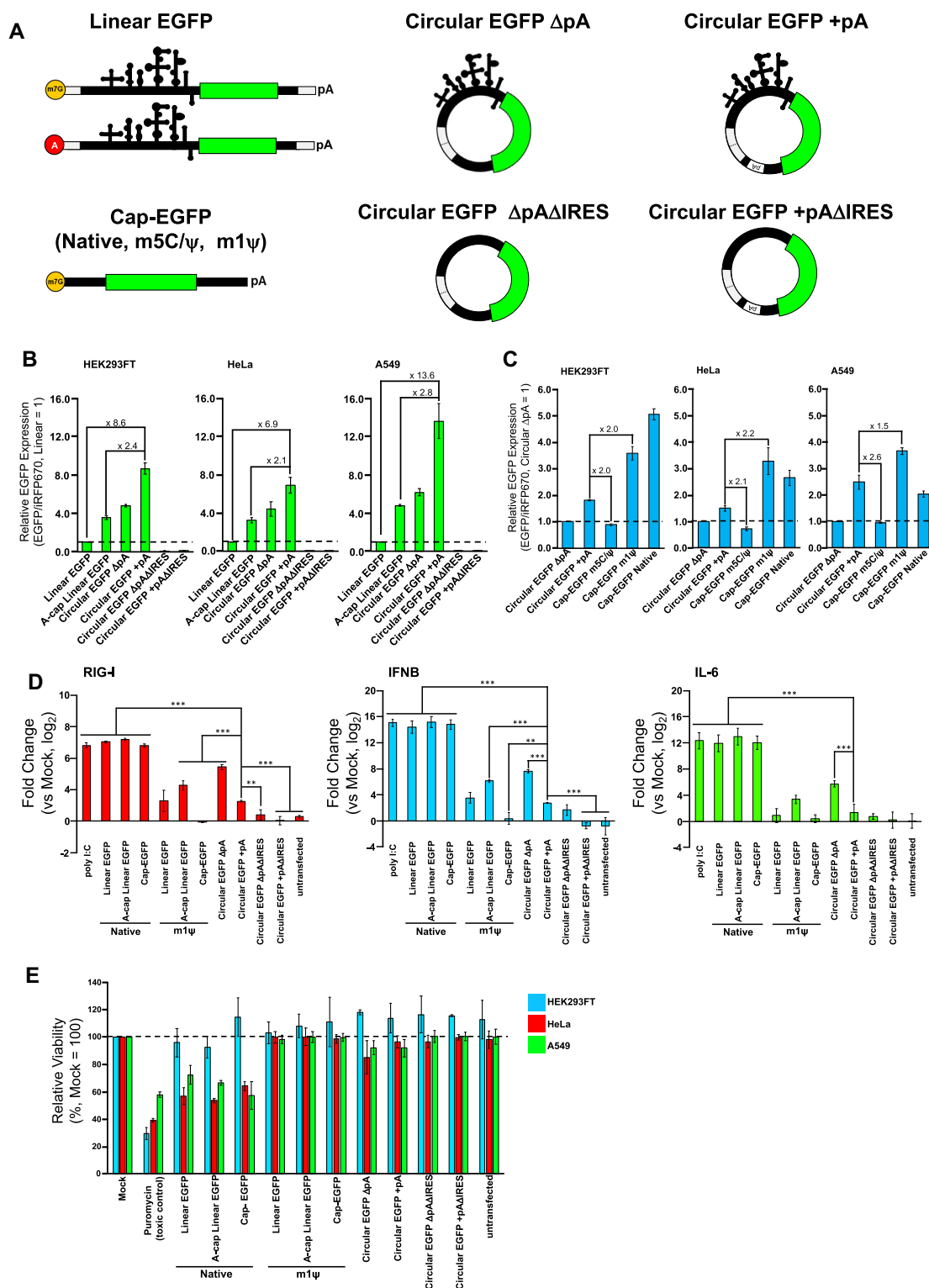


Figure 3. Comparison of reporter expression and immunogenicity. (A) Structure illustration of linear mRNAs and circRNAs used in these evaluations. All mRNAs code *EGFP* as a reporter gene. (B) Comparison of reporter expression between circRNAs and linear mRNAs with the same sequence component. 0.3 pmol of each reporter mRNA and transfection control, *iRFP670*-coding mRNA were transfected. (C) Comparison of circRNAs and linear mRNAs (Cap-EGFPs) without any other structural features corresponding to CVB3 IRES and self-splicing ribozyme. 0.3 pmol of each reporter *EGFP*-coding mRNA and transfection control, *iRFP670*-coding mRNA were transfected. (D) Evaluation of immune response-related genes by RT-qPCR after 24 h of transfection in A549 cells. 0.6 pmol of each mRNA was transfected. 200 ng poly I:C was transfected as a positive control. Levels of significance are denoted as $^{**}P < 0.01$, $^{***}P < 0.001$ (Dunnett's test). N.S. (non-significant, $P > 0.05$) pairs were not denoted on the graph. (E) Evaluation of cytotoxicity by WST-1 assay was performed with a 96-well format. 0.15 pmol of each mRNA was transfected. For toxic control, the cells were cultured in 1 μ g/ml (HEK293FT, HeLa) or 2 μ g/ml (A549) puromycin for 1 day before the measurement. All data in this figure are presented as mean $\pm$ SD, $n = 3$.

First, we compared EGFP expression of circRNA and linRNA with the same sequence components (UTR, CVB3 IRES, ribozyme-derived sequence, *EGFP*-coding region, pA). circRNAs with IRES showed higher expression levels than linRNAs in the three tested human cell lines (HEK293FT, HeLa, A549) whereas circRNAs Δ IRES did not activate translation (Figure 3B). Notably, circRNA + pA showed higher EGFP expression than IRES-dependent linRNAs or circRNA Δ pA (Figure 3B, Supplementary Figure S2A), confirming that the pA sequence enhances protein expression from circRNA. It has been reported that pA or pAC spacer sequences inserted to the vicinity of 5' and 3', or only that of 5' intron fragments in circRNA promote translation by interaction with eukaryotic translation initiation factor (eIF4G) and polyA-binding protein (PABP), similar to the translation mechanism of linRNA (3,40,41). We assume that a similar effect was also observed when the 120-mer polyA sequence used in the tail of our linRNA was inserted internally into the circRNA (between the 3' UTR and the 3' intron fragment). To investigate the stabilization of RNA by cyclization, we quantified the amount of residual circRNA and linRNA by RT-qPCR. The relative levels of circRNA (Circular EGFP + pA) are higher than linRNA (Linear EGFP) at 8 and 24 h after transfection (Supplementary Figure S2B), indicating that enhanced stability by cyclization is likely to contribute to higher reporter expression.

We next compared the performance of circRNAs with conventional cap-dependent linRNAs (Cap-EGFP) with modified bases (m5C/ ψ , m1 ψ ; modRNAs). These base modifications have been applied to synthetic mRNAs to reduce immunogenicity and improve protein expression (42–44). Although the protein expression level from circRNA + pA was lower than m1 ψ or native linRNA, it showed a higher expression level than that from Cap-EGFP with m5C/ ψ (Figure 3C, Supplementary Figure S2C). The results indicate that the expression level from circRNA is superior to that from linRNAs with the same sequence context and cap-dependent modRNA with m5C/ ψ .

We next investigated the effect of immunogenicity of linRNAs or circRNA transfection by RT-qPCR (Figure 3D). As expected, expression levels of immune response-related genes (RIG-1, IFNB and IL-6) were upregulated after transfecting native mRNA, and the degree of upregulation was decreased when the modRNA (m1 ψ) equivalent was transfected in place of its native mRNA. We observed that circRNA constructs with native bases also reduced the expression of these genes, confirming the reduced immunogenicity of circRNAs (16). CircRNA Δ pA showed similar levels of the expression of these genes compared with Acap-linRNA (m1 ψ). Interestingly, circRNA + pA decreased the expression of these genes compared with circRNA Δ pA, indicating that the addition of the internal polyA sequence further reduced the immunogenicity of circRNA.

Additionally, we investigated the cytotoxic effect of the circRNAs in the three cell lines (Figure 3E). We confirmed that transfection of linRNA with native base decreased HeLa or A549 cell viability whereas that of modRNA (m1 ψ) maintained normal cell viability, confirming previous observations (42–44). Our circRNAs (circRNA + pA and circRNA Δ pA, with or without IRES sequences) also

maintained cell viability without cytotoxic effect. Thus, we conclude that our circRNA constructs efficiently expressed target proteins without inducing severe cell cytotoxicity and immunogenicity, which can be used for further circRNA engineering.

Construction and evaluation of miRNA-responsive circRNA switches

Next, we designed circRNAs that contained a fully complementary anti-miR sequence at the UTRs and evaluated them by co-transfecting various miRNA mimics. We prepared four different *Homo sapiens* microRNA (hsa-miR-206, hsa-miR-302a-5p, hsa-miR-21-5p and hsa-miR-339-5p)-responsive circRNA switches by inserting anti-miR either before the CVB3 IRES (5'-insertion) or after the *EGFP*-coding sequence (3'-insertion). Twenty-four h after transfection with circRNA switches and the corresponding miRNA mimics, we analyzed EGFP expression from the circRNAs by flow cytometer and fluorescent microscopy. Notably, all designed miRNA-responsive switches repressed EGFP expression by sensing the target miRNA mimic (Figure 4A, Supplementary Figure S3A). The observed fold changes (approximately 2- to 38-fold ranges) between the ON state and OFF state depended on the target miRNA, the insertion position of anti-miR, and the presence or absence of an internal polyA sequence (Figure 4B).

We speculated that the relatively low-fold change of miR-21-5p- and miR-339-5p-responsive switches might be due to endogenous miRNA activity expressed in the HEK293FT cell (45). Thus, we added target miRNA inhibitors into the cells to block the activity of endogenous miRNA. Co-transfection with the miR-21-5p inhibitor resulted in the rescue of circRNA translation; however, this was not seen for the miR-339-5p inhibitor (Supplementary Figure S3B). This result suggests that the observed low fold change in the case of the miR-21-5p-responsive switch is due to a decrease in the ON state caused by intrinsic miR-21-5p activity. In fact, our previous study showed that the HEK293FT cell expresses endogenous miR-21-5p, although its activity is lower compared with other cancer cell lines (e.g. HeLa cells) (44,45). For the miR-339-5p-responsive switch, however, it may be caused by other factors like accessibility to the target site caused by RNA secondary structures, as predicted with CentroidFold (46) (Supplementary Figure S3C).

To investigate whether target miRNA could enhance the cleavage and degradation of miRNA-sensing circRNAs, we analyzed circRNA levels in the presence or absence of miRNA mimic by RT-qPCR. We used miR-206- and miR-302a-5p-responsive switches and the corresponding primer pairs that amplify the remaining sequence at three different regions of the circRNAs (Supplementary Figure S4A). The presence of target miRNA mimic enhanced the degradation of both circRNA switches, confirming the miRNA-mediated circRNA degradation. The surrounding region of the miRNA target site in the 3' UTR (ORF end~3UTR) is more susceptible to degradation than the top and middle region of ORF (ORF top, ORF middle) (Supplementary Figure S4B), suggesting that miRNA-responsive circRNA switch may undergo endonucleolytic cleavage at the fully matched miRNA target site portion and be degraded from

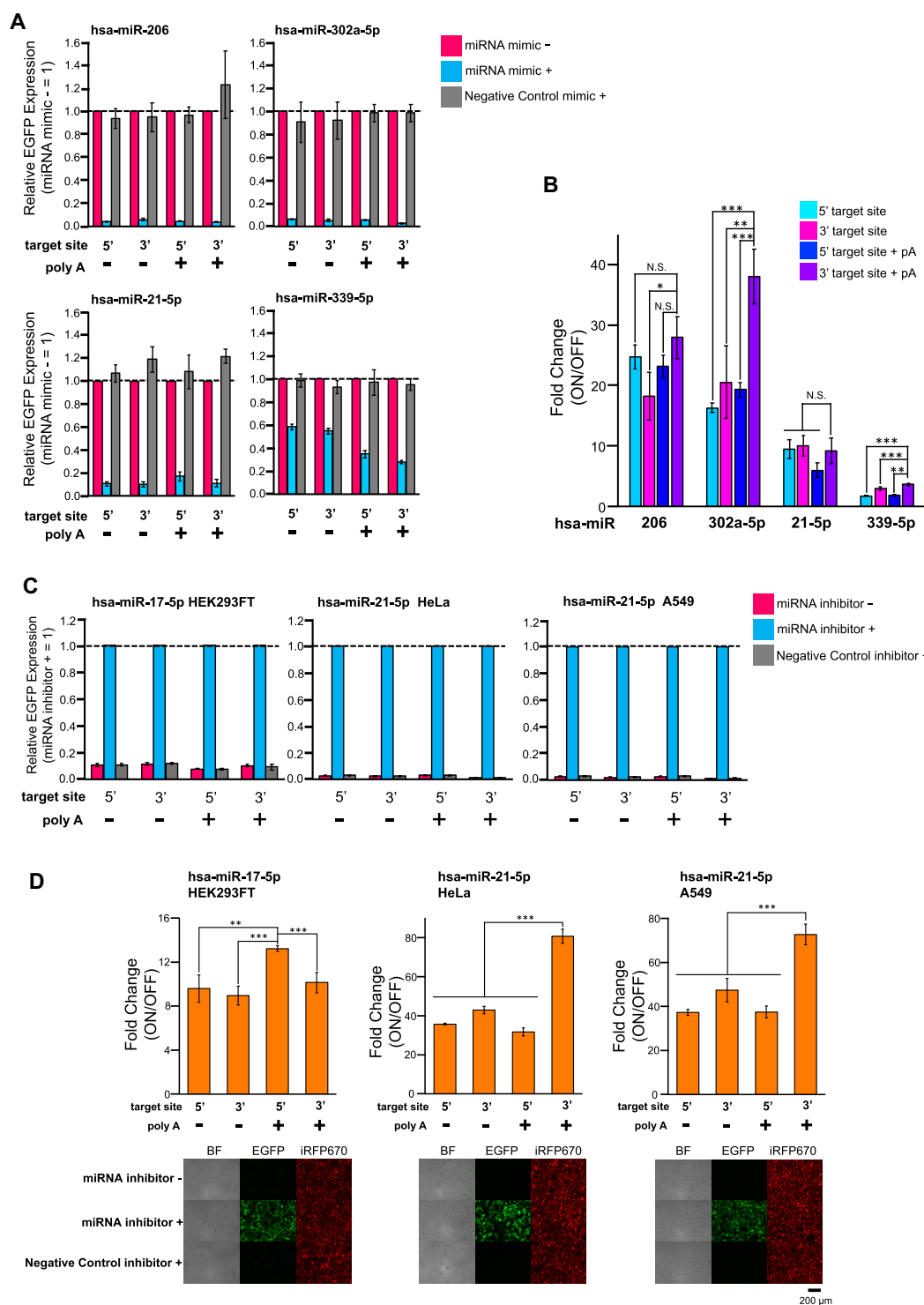


Figure 4. Design and evaluation of miRNA-responsive circRNA switches. (A) Evaluation by co-transfecting miRNA mimics in HEK293FT cells. Relative EGFP Expression was calculated by normalizing the sample without a miRNA mimic (magenta). 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected. 0.25 pmol of specific miRNA mimic or Negative Control mimic was co-transfected for evaluation. (B) The fold-change of each miRNA-responsive circRNAs was evaluated in (A). The ON state was determined as the sample without miRNA mimic. (C) Endogenous miRNA detection by miRNA-responsive circRNAs in HEK293FT, HeLa and A549. Relative EGFP Expression was calculated by normalizing the sample with a specific miRNA inhibitor (cyan). 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected. 1 pmol (hsa-miR-17-5p, HEK293FT), 2 pmol (hsa-miR-21-5p, HeLa) or 4 pmol (hsa-miR-21-5p, A549) of specific miRNA inhibitor or Negative Control inhibitor was co-transfected for evaluation. (D) The fold-change of each endogenous miRNA-responsive circRNA was evaluated in Figure 4C and fluorescent images of miRNA-responsive circRNA switches showing the best fold change. The ON state was determined as the sample with a specific miRNA inhibitor. The scale bar at the fluorescent images indicates 200 μ m. Levels of significance are denoted as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Dunnett's test). N.S. means non-significant ($P > 0.05$). All data in this figure are presented as mean $\pm$ SD, $n = 3$.

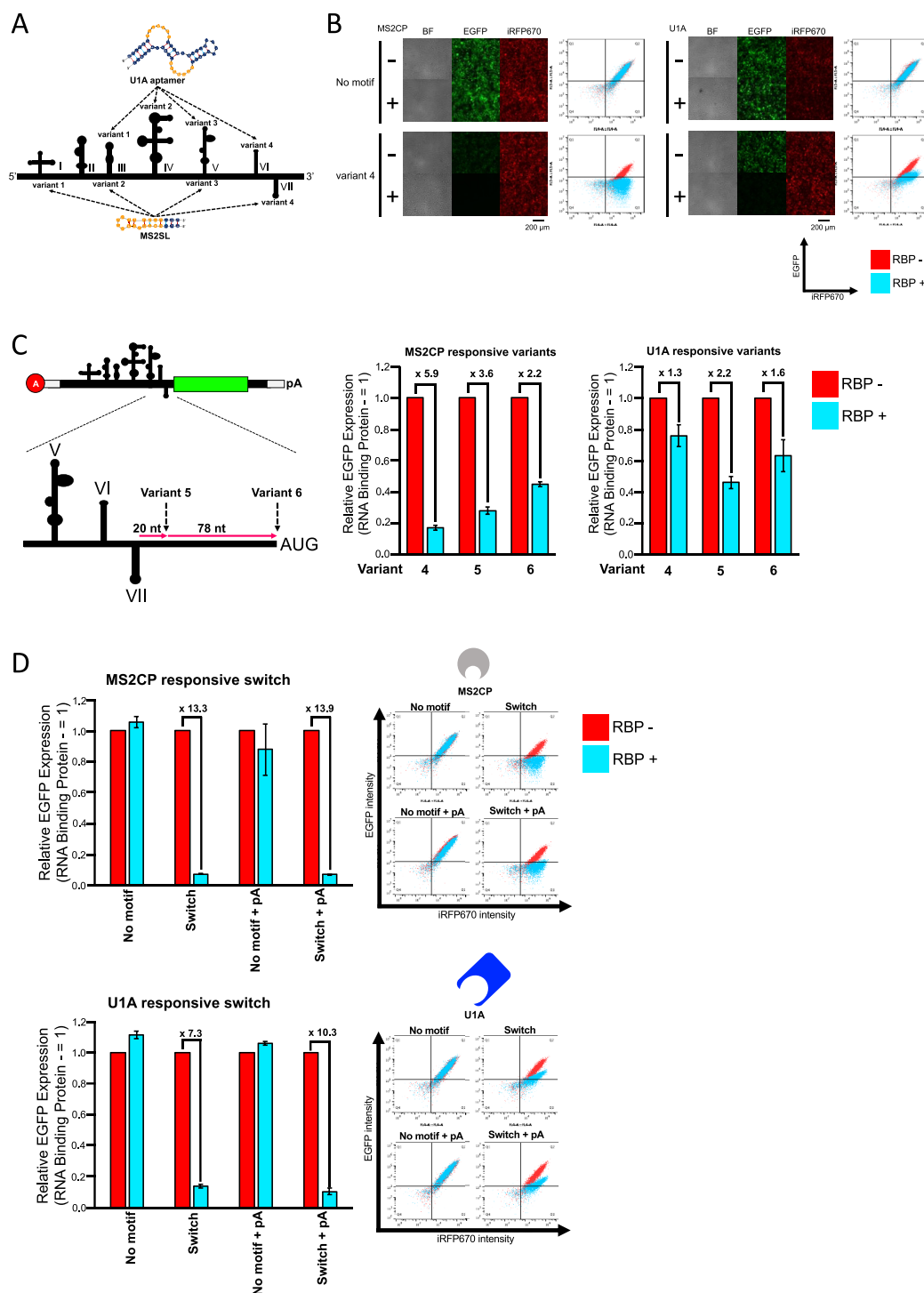


Figure 5. Design and evaluation of protein-responsive circRNA switches. (A) Investigation of effective position for the protein-binding motif (aptamer) insertion. Four different variants (variants 1–4) were designed by inserting MS2SL or U1A aptamer. Orange bases in MS2SL and U1A aptamer indicate that protein-binding region. (B) Fluorescent microscope images and scatter plots of flow cytometry evaluated by co-transfecting MS2CP or U1A-coding mRNA in HEK293FT. The scale bar at the fluorescent images indicates 200 μ m. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected. 0.05 pmol of RBP (MS2CP or U1A)-coding mRNA was co-transfected for evaluation. (C) Optimization of the position for the motif-insertion. Additional variants were designed by inserting the motif at 20 nt (variant 5) or 98 nt (variant 6) downstream of the domain VII stem-loop. A-cap Linear EGFP with the variants was used for evaluation. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected. 0.05 pmol of RBP (MS2CP or U1A)-coding mRNA was co-transfected for evaluation. (D) Evaluation of U1A and MS2CP-responsive circRNA switches by using circRNA contexts in HEK293FT cells. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected. 0.05 pmol of MS2CP-coding mRNA or 0.15 pmol of U1A-coding mRNA was co-transfected for evaluation. All data in this figure are presented as mean $\pm$ SD, $n = 3$. Relative EGFP Expression was calculated by normalizing the sample without target protein-coding mRNA. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670.

the vicinity of the target site after the miRNA-mediated cleavage (20,29). The results also indicate that the low leakage expression observed in the OFF state of the circRNA switch (Supplementary Figure S3A, mimic+) may be due to the translation from partially degraded circRNAs.

In addition, we investigated whether the designed circRNA switches could detect endogenous miRNAs and modulate translation in the target cell. We focused on hsa-miR-17-5p in HEK293FT and hsa-miR-21-5p in HeLa and A549 because these miRNAs are efficiently expressed in each cell type (45,47). The reporter expression from the transfected switches was rescued only when it was co-transfected with a target miRNA-specific inhibitor, showing ON/OFF fold changes were approximately 12-fold (hsa-miR-17-5p) and 80-fold (hsa-miR-21-5p), with clear separation of each cell with or without the inhibitor by flow cytometry (Figure 4C, D, and Supplementary Figure S5), which was comparable to the results obtained with the miRNA mimics (Figure 4A, B, and Supplementary Figure S3A). These results indicate that miRNA-responsive circRNA switches efficiently detected target miRNAs, controlling their translation level in a cell type-specific manner.

CVB3 IRES engineering for RBP-responsive circRNA switches

We next designed protein-responsive circRNA by using two RBPs: MS2 bacteriophage coat protein (MS2CP) and spliceosome-related SNRPA (U1A) protein. To investigate the positions of protein-binding that enable IRES-dependent translation repression, we tested four CVB3 IRES variants (variants 1–4) designed by inserting protein-binding motifs (MS2SL and U1A aptamer) while referring to the secondary structure model and structural features necessary for translation initiation (32,48,49) (Figure 5A). Inserting the MS2CP or U1A-binding motif showed that only variant 4, in which the motif was inserted in domains VI (MS2CP) or VII (U1A), was able to repress translation in the presence of the target RBP, whereas other variants did not efficiently express the reporter or did not respond to target RBP (Figure 5B and Supplementary Figure S6).

From these results, we expected that motif insertion to CVB3 IRES near or downstream of the binding site for translation initiation factors, eIF4G and eIF4A (which bind to domains V–VII) (49), or the antisense region against 18S rRNA (the linker region between domains V and VI) (48), may be effective for generating the RBP-responsive circRNA switch. To optimize the insertion position, we further designed variants 5 and 6, with the inserted-motif at 20 nt or 98 nt (just upstream of the ORF) downstream of the domain VII stem-loop and compared their repression efficiencies with that of variant 4. We evaluated the protein production of CVB3 IRES variants 4–6 using A-cap linear *EGFP* mRNA which also functioned as a miRNA-responsive switch (Supplementary Figure S7). The results showed that variant 4 for MS2SL, and variant 5 for U1A aptamer were the best for translation repression in the presence of MS2CP or U1A (Figure 5C and Supplementary Figure S8). We applied these variants to evaluate their performance in circRNA to confirm the effect of the presence or absence of the internal polyA sequence on translational

suppression and fold change. The translational repression observed in circRNA switch constructs was all efficient, as seen in the fold changes (7.3- to 13.9-fold ranges) between ON and OFF states in the presence or absence of MS2CP or U1A (Figure 5D). Together, these results indicate that RBP-responsive circRNA switches can be designed by engineering the regions of domains VI and VII of CVB3 IRES.

In addition, we analyzed the expression level of MS2CP and U1A by western blotting and confirmed the effective expression from these RBP-coding mRNAs (Supplementary Figure S9A and B). Although we detected the expression of endogenous U1A, an apparent increase in expression was observed in cells transfected with *U1A*-coding mRNA. We next investigated whether endogenous U1A protein may affect the ON state of U1A-responsive circRNA, even in the absence of *U1A*-coding mRNA. We performed a knock-down assay of endogenous U1A by shRNAs used in the previous study (30). As expected, the endogenous U1A expression was repressed by U1A-targeted shRNA (Supplementary Figure S9C, left), but the knockdown of endogenous U1A did not increase the reporter EGFP expression from the U1A-responsive circRNA (Supplementary Figure S9C, right), suggesting that endogenous U1A does not affect the performance of the circRNA switch under the condition.

Construction of synthetic circRNA circuits and their extended driving

Finally, we investigated whether circRNAs could improve the driving time of synthetic RNA circuits composed of both the miRNA-responsive and RBP-responsive switches. When multiple switches are available, it is possible to construct synthetic genetic circuits by designing the output from one switch to be the input of another switch (23) (Figures 1B and 6A).

We first confirmed the enhanced stability and gene expression persistence of the designed circRNA. We compared gene expressions of *MetLuc2* luciferase (*MetLuc2*-coding circRNA (Circular *MetLuc2* + pA), with conventional cap-dependent linRNAs with modified bases (m5C/ψ, m1ψ) using the three human cell lines (Supplementary Figure S10). The circRNA constructs showed better expression persistence than the linRNAs with enhanced expression duration by modified bases (42), consistent with the previous reports (3,16).

We next designed a miRNA-responsive ON system by using two circRNA switches, miRNA (miR-302a-5p)-responsive circRNA that produced RBP (MS2CP or U1A) and the RBP-responsive circRNA that produced EGFP. The designed circRNA circuits using either MS2CP or U1A produced EGFP only in the presence of miR-302a-5p mimic, functioning as an ON switch by detecting target miRNA (Figure 6B, left). The fold changes between the ON and OFF states of these miRNA-responsive circuits were approximately 3.5-folds in the presence of 1 pmol of miR-302a-5p mimic (Figure 6B, right).

We also compared the performance of the persistence of circRNA circuits with linear mRNA circuits used in previous studies (31,44,50), by using the *MetLuc2* reporter. We tested them using two cell lines, HEK293FT and A549, to detect exogenous miR-302a-5p mimic or endogenous

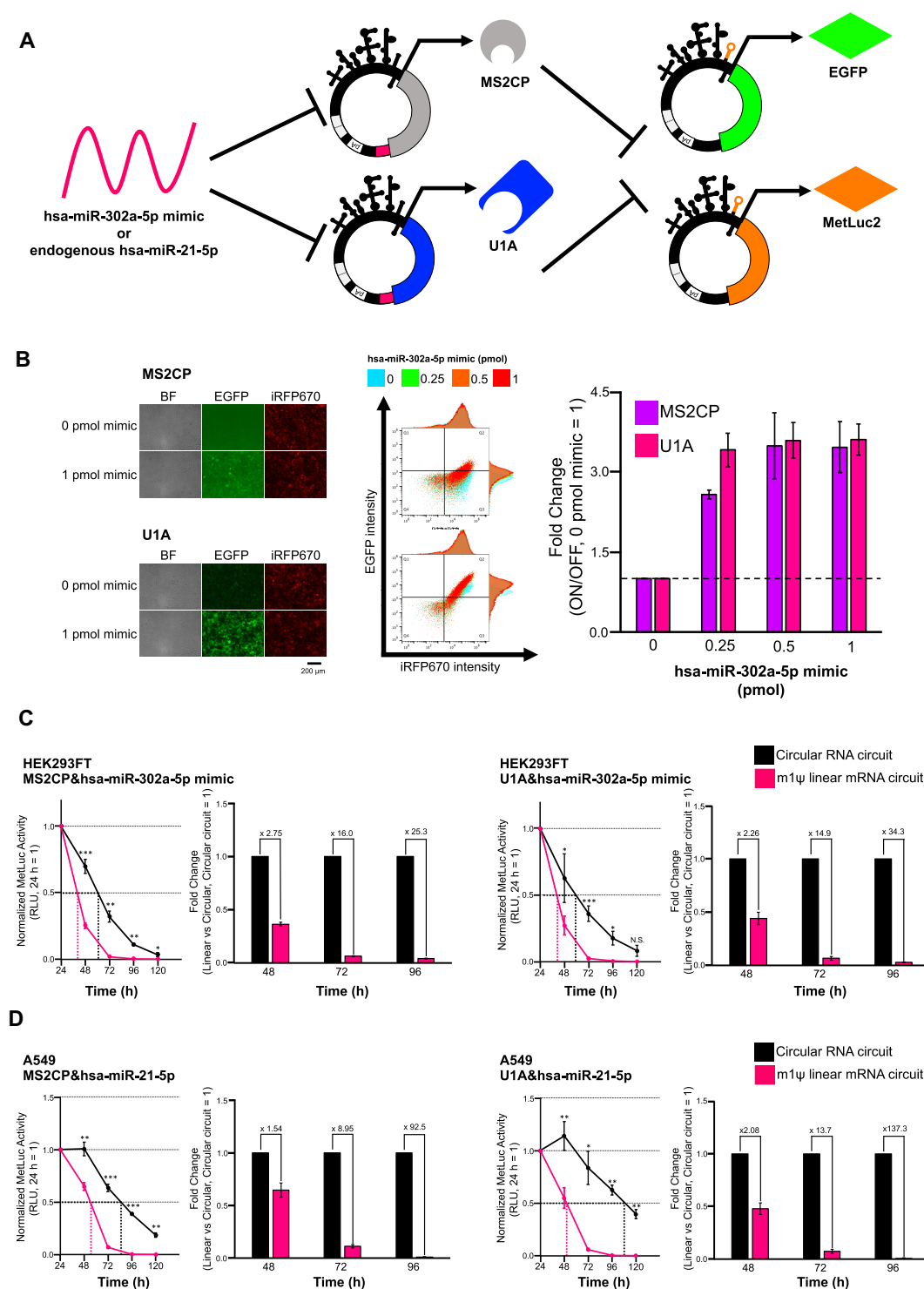


Figure 6. circRNA-based gene circuits that activate translation by detecting miRNA. (A) Schematic illustration of miRNA-responsive ON circuit. MS2CP or U1A is encoded on the first miRNA-responsive circRNA switch. The reporter gene (*EGFP* or *MetLuc2*) is encoded on the second protein-responsive circRNA switch. (B) Fluorescent images, scatter plots of a flow cytometer, and titration results of ON circuits with miR-302a-5p mimic in HEK293FT cells. The scale bar at the fluorescent images indicates 200 μ m. 0.3 pmol of RBP (MS2CP or U1A)-coding mRNA, reporter mRNA and transfection control mRNA were transfected. 0.25, 0.5 or 1 pmol of hsa-miR-302a-5p mimic was co-transfected for evaluation. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670. (C) Evaluation of circRNA circuit persistence with a miR-302a-5p mimic in HEK293FT cells. 45 fmol of RBP (MS2CP or U1A)-coding mRNA and reporter mRNA were transfected. 1 pmol of hsa-miR-302a-5p mimic was co-transfected to activate the circuits. (D) Evaluation of circRNA circuit persistence with endogenous miR-21-5p in A549 cells. 45 fmol of RBP (MS2CP or U1A)-coding mRNA and reporter mRNA were transfected. Levels of significance are denoted as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (two-tailed unpaired Student's or Welch's t -test determined by F -test). N.S. means non-significant ($P > 0.05$). All data in this figure are presented as mean $\pm$ SD, $n = 3$.

miR-21-5p, respectively. In both cases, the circRNA circuit showed better performance of duration than the linear RNA circuit with m1 ψ (1.5- to 137-fold duration) after approximately 96 h of RNA transfection (Figure 6C and D), indicating that our circRNA circuits improved the expression persistence of synthetic mRNA circuits.

DISCUSSION

In this study, we provided the principle for designing miRNA and protein-responsive circRNA switches with controlled expression by detecting intracellular conditions in mammalian cells. No previous studies have shown synthetic circRNAs with regulatory functions. In particular, RBP-mediated translational repression of circRNA was not observed in either the synthetic or endogenous system. Among RBP-responsive CVB3 IRES variants, only those in which the protein-binding motif was inserted into domain VI or VII, downstream of the binding site for translation initiation factors and antisense region against 18S rRNA, were functional (variant4, Figure 5A, B and Supplementary Figure S6). A previous report with a cap-dependent system suggested that inhibition of the translation initiation step by RBP binding plays a central role in repression (51,52). Our results indicate that the protein-responsive switch can also be designed for cap-independent translation by inserting motifs at a position that efficiently inhibits the assembly of the translation initiation factors or subsequent ribosome scanning in the IRES. The suitable position for the motif insertion seems to be located at the downstream region of domain VI in the CVB3 IRES, which was confirmed by experiments using variants 1–6 (Figure 5B, C and Supplementary Figure S6). Further investigations will be required to study whether similar results could be obtained in other IRES-inserted circRNA switches in the future. For miRNA-responsive circRNA switches, it was noted that they were able to detect endogenous miRNAs in the target cell (hsa-miR-17-5p in HEK 293FT, hsa-miR-21-5p in HeLa and A549 cells), making it possible to regulate circRNA translation in a cell-type-specific manner (Figures 4D and 6D). Such cell-type-specific regulation would reduce potential side effects for future mRNA therapeutics.

Due to the superior stability and expression persistence of circRNAs, RNA-based gene circuits constructed from circRNA switches were more durable than those composed of linear modRNAs (Figure 6 and Supplementary Figure S10). Thus, our circRNA switches may solve the previously reported issue of a shorter half-life for modRNA-based circuits (23). Durable RNA-based gene circuits have also been realized using replicon vectors (23). Our circRNA-based circuits may have several advantages over such replicon-based circuits, such as a more compact size, no unexpected self-replication, easier handling, and higher transfection efficiency with lipid nanoparticle-based systems (53). Direct comparison of the performances between circRNA- and replicon-based circuits will be important in future studies.

Improving the performance of circRNA switches should also be addressed in future studies. In the comparison using our linear mRNA switch systems (31,44,50) (linear switches) with HEK293FT, the performance of miRNA-responsive circRNA switches was comparable (m1 ψ) or superior (m5C/ ψ) to that of base-substituted linear switches,

except for the one affected by endogenous miR-21-5p activity (Supplementary Figure S11A), whereas protein-responsive circRNA switches performed less efficiently than m1 ψ -substituted linear mRNA switches (Supplementary Figure S11B). In the linear switches, it has been shown that incorporation of m1 ψ to mRNA could enhance its sensitivity to several miRNAs and RBPs, contributing to better performance (44,54). However, CVB3 IRES with the base modification (e.g. m5C/ ψ or m1 ψ) disrupted protein expression (16) (Supplementary Figure S12). Therefore, to develop chemically modified IRES-dependent circRNA switches, we need to engineer functional IRES with modified bases through rational engineering or directed evolution approaches.

RNA sequence and structure engineering is an alternative method to achieve a superior ON and OFF state without modified-base substitution for circRNA switches. Previous research on linear mRNA engineering has achieved improvements through codon optimization of the ORF (55), more stable UTRs (56,57), and engineering for protein or miRNA binding sequences (30,31,58). These approaches may be adaptable to circRNA-based systems, although optimization would be required in some cases. For example, in miRNA-responsive circRNA switches, they likely preferred 3' insertion, rather than 5', with internal polyA-containing constructs (Figure 4B), contrary to our previous observation with the linear mRNA system (22). We expected this tendency was caused by the rigid structure derived from CVB3 IRES on the 5' side, which may affect miRNA-mediated circRNA degradation, although the miR-17-5p-responsive circRNA switch showed the best fold-change in 5' target site insertion (Figure 4D). In addition, contrary to the previous report of increased sensitivity with increasing copy number of miR-302a-5p target sites in the linear mRNA switch (58), a single copy insertion in the 3' UTR showed the best fold-change between ON and OFF states for miR-302a-5p responsive circRNA switch (Supplementary Figure S13, right). In the case of the miR-206-responsive circRNA switch, 2-copy insertion into both the 5' and 3' UTRs (2 $\times$ 2 insertion, 4 copies total) showed the best fold-change (Supplementary Figure S13, left). These observations suggest that sequence dependency in circRNA systems is more pronounced than in linRNA systems, and that target miRNA-specific optimization steps will be required to maximize performance for circRNA switches (59). Recently, Chen et al. reported the sequence elements that enhance circRNA performance (41). The identified various accessory parts and engineered IRES with enhanced translation would improve the performance of circRNA switch (e.g. enhanced ON state) (44).

Recently, it has been reported that exogenous circRNAs cause an immune response, while contrasting reports suggested that they are less immunogenic (15–17). RT-qPCR analysis for immune response-related genes (RIG-I, IFNB, IL-6) showed that our circRNA is less immunogenic than linear mRNAs with native bases, whereas a more intense immune response was observed compared with chemically modified linear mRNAs, especially linear mRNAs with the commonly used Cap-EGFP structure (Figure 3D). Notably, lower immunogenicity was observed in circRNAs with internal polyA sequences compared to those without an internal polyA sequence. A previous report by Liu *et al.* sug-

gested that the RNA duplex constructed by ribozymes and CVB3 IRES causes circRNA immunogenicity (18). Our observations indicate that immunogenicity caused by such structures in circRNAs may be reduced by internal polyA-120 sequence longer than constructs in previous reports (3,16,41). Thus, the stronger expression levels observed with long polyA insertion may be a synergistic effect of the recruitment of eIF4G by PABP (3,40,41) and the reduction of immunogenicity. We expect that further investigation is needed to determine the composition and length of the polyA sequence to achieve enhanced translation and reduced immunogenicity. In terms of cytotoxicity, our circRNAs showed better cell viability than native linear mRNAs, which was comparable to those of chemically modified ones (Figure 3E), indicating that the induction level of these immune response-related genes by our circRNA does not affect cells. However, future *in vivo* evaluation is required to determine how these immunogenicity differences will affect actual therapeutic applications.

In conclusion, our circRNA switches and circuits provide new insights into the engineering of circRNA, which is still underdeveloped compared to linear synthetic mRNA. We believe that synthetic circRNA devices with translation regulations process the broad potential for synthetic biology, mRNA-based therapies, and cellular engineering.

DATA AVAILABILITY

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information files. All raw data for each graph are shown in the Supplementary Table S3. Requests for materials should be made to the corresponding author. All plasmids and mRNAs generated in this study are available upon request.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Synthetic circular RNA switches and circuits that control protein expression in mammalian cells

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Supplementary Sequences

Supplementary References

Supplementary Tables

Supplementary Table S1. Transfection overview

Figure	Transfected-mRNA(s) (/well)	miRNA mimic/inhibitor (/well)	Cell line and number of seeded cells (cells/well)	Lipofectamine MessengerMAX (μl/well)
3B	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol Linear EGFP (m7G cap, A-cap), Circular EGFP (DpA, +pA) or Circular EGFP DIRES (DpA, +pA)	—	HEK293FT, A549 : 1.0 x 10 ⁵ HeLa : 0.5 x 10 ⁵	1
3C	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol Circular EGFP DpA, Circular EGFP +pA or Cap-EGFP (Native, m5C/Ψ, m1Ψ)	—	HEK293FT, A549 : 1.0 x 10 ⁵ HeLa : 0.5 x 10 ⁵	1
3D	0.6 pmol Linear EGFP (m7G-cap, A-cap, Native, m1Ψ), Circular EGFP (DpA, +pA) Circular EGFP DIRES (DpA, +pA) or Cap-EGFP (Native, m1Ψ)	—	A549 : 1.0 x 10 ⁵	1
3E	0.15 pmol Linear EGFP (m7G-cap, A-cap, Native, m1Ψ), Circular EGFP (DpA, pA+) Circular EGFP DIRES (DpA, pA+) or Cap-EGFP (Native, m1Ψ)	—	HEK293FT, A549 : 2.0 x 10 ⁴ HeLa : 1.0 x 10 ⁴	0.2
4A · B	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol 5' or 3'T miR Circular EGFP DpA or 5' or 3'T miR Circular EGFP +pA 5' or 3'T miR Linear EGFP	0.25 pmol miRNA mimic or Negative Control mimic	HEK293FT : 1.0 x 10 ⁵	1
4C · D	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol 5' or 3'T miR Circular EGFP DpA or 5' or 3'T miR Circular EGFP +pA	HEK293FT : 1 pmol miR-17-5p inhibitor or Negative Control inhibitor HeLa : 2 pmol miR-21-5p inhibitor or Negative Control inhibitor A549 : 4 pmol miR-21-5p inhibitor or Negative Control inhibitor	HEK293FT, A549 : 1.0 x 10 ⁵ HeLa : 0.5 x 10 ⁵	1
5B	0.3 pmol Cap-iRFP670 (m5C/Ψ) MS2CP : 0.3 pmol Circular EGFP or MS2CP responsive Circular EGFP variant1~4, 0.05 pmol Cap-MS2CP (m1Ψ) U1A : 0.3 pmol Circular EGFP or U1A responsive Circular EGFP variant1~4, 0.05 pmol Cap-U1A (m1Ψ)	—	HEK293FT : 1.0 x 10 ⁵	1
5C	0.3 pmol Cap-iRFP670 (m5C/Ψ) MS2CP : 0.3 pmol MS2CP responsive linear EGFP variant4 ~6 (A-cap), 0.05 pmol Cap-MS2CP (m1Ψ) U1A : 0.3 pmol U1A responsive linear EGFP variant4~6 (A-cap), 0.05 pmol Cap-U1A (m1Ψ)	—	HEK293FT : 1.0 x 10 ⁵	1
5D	0.3 pmol Cap-iRFP670 (m5C/Ψ) MS2CP : 0.3 pmol Circular EGFP (DpA or pA+) or MS2CP responsive Circular EGFP variant 4 (DpA or pA+), 0.05 pmol Cap-MS2CP (m1Ψ) U1A : 0.3 pmol Circular EGFP (DpA or pA+) or U1A responsive Circular EGFP variant 5 (DpA or pA+), 0.15 pmol Cap-U1A (m1Ψ)	—	HEK293FT : 1.0 x 10 ⁵	1
6B	0.3 pmol Cap-iRFP670 (m5C/Ψ) MS2CP : 0.3 pmol MS2CP responsive Circular EGFP variant4 +pA, 0.3 pmol 3'T302a-5p Circular MS2CP +pA U1A : 0.3 pmol U1A responsive Circular EGFP variant5 +pA, 0.3 pmol 3'T302a-5p Circular U1A +pA	0, 0.25, 0.5 1 pmol miR-302a-5p mimic	HEK293FT : 1.0 x 10 ⁵	1
6C	MS2CP : 45 fmol MS2CP responsive Circular MetLuc2 variant4 + pA, 45 fmol 3'T302a-5p Circular MS2CP +pA U1A : 45 fmol U1A responsive Circular EGFP variant5 +pA, 45 fmol 3'T302a-5p Circular U1A + pA	1 pmol miR-302a-5p mimic	HEK293FT : 1.5 x 10 ⁴	1
6D	MS2CP : 45 fmol MS2CP responsive Circular MetLuc2 variant4 +pA, 45 fmol 3'T21-5p Circular MS2CP +pA U1A : 45 fmol U1A responsive Circular EGFP variant5 +pA, 45 fmol 3'T21-5p Circular U1A +pA	—	A549 : 1.5 x 10 ⁴	1

Figure	Transfected-mRNA(s) (/well)	miRNA mimic/inhibitor (/well)	Cell line and number of seeded cells (cells/well)	Lipofectamine MessengerMAX (μl/well)
S2B	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol Linear EGFP (m7G cap) or Circular EGFP (+pA)	—	HEK293FT : 1.0 x 10 ⁵	1
S3B	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol 5' or 3'T miR Circular EGFP or 5' or 3'T miR Circular EGFP +pA	0.25 pmol miRNA mimic, Negative control mimic, miRNA inhibitor or Negative Control inhibitor	HEK293FT : 1.0 x 10 ⁵	1
S4B	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol 5' or 3'T miR Circular EGFP +pA	0.25 pmol miRNA mimic or Negative Control mimic	HEK293FT : 1.0 x 10 ⁵	1
S7	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol 5' or 3'T miR Linear EGFP or 5' or 3'T miR Circular EGFP DpA or 5' or 3'T miR Circular EGFP +pA	0.25 pmol miRNA mimic	HEK293FT : 1.0 x 10 ⁵	1
S9A · B	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol Circular EGFP +pA 0.05 pmol Cap-MS2CP (m1Ψ) or 0.15 pmol Cap-U1A (m1Ψ)	—	HEK293FT : 1.0 x 10 ⁵	1
S9C	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol U1A responsive Circular EGFP variant5 +pA 1, 2, 4, pmol shRNA (U1A or control)	—	HEK293FT : 1.0 x 10 ⁵	1
S10B	45 fmol Circular MetLuc2 +pA or Cap-MetLuc2 (m5C/Ψ, m1Ψ)	—	HEK293FT, A549 : 1.5 x 10 ⁴ HeLa : 0.5 x 10 ⁴	1
S11A	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol 3'T miR Circular EGFP +pA or linear miRNA-responsive switch (m5C/Ψ, m1Ψ)	0.25 pmol miRNA mimic	HEK293FT : 1.0 x 10 ⁵	1
S11B	0.3 pmol Cap-iRFP670 (m5C/Ψ) MS2CP : 0.3 pmol MS2CP responsive Circular EGFP variant4 +pA or 0.3 pmol EGFP linear switch (m5C/Ψ, m1Ψ), 0.05 pmol Cap- MS2CP (m1Ψ) U1A : 0.3 pmol U1A responsive Circular EGFP variant5 + pA or 0.3 pmol EGFP linear switch mRNA (Native, m5C/Ψ or m1Ψ), 0.15 pmol Cap-U1A (m1Ψ)	—	HEK293FT : 1.0 x 10 ⁵	1
S12	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol Linear EGFP (Native, m5C/Ψ, m1Ψ)	—	HEK293FT : 1.0 x 10 ⁵	1
S13	0.3 pmol Cap-iRFP670 (m5C/Ψ) 0.3 pmol 3'T, 4x 5'T, 4x3'T or 2x2 5&3'T miR Circular EGFP +pA	0.125, 0.25. 0.5 pmol miRNA mimic	HEK293FT : 1.0 x 10 ⁵	1

All experiments were performed in 24-well format, except for Figure 3E (WST-1 assay), which was performed in 96-well format.

Supplementary Table S2. Primers used in RT-qPCR analysis.

Target gene	Primers (5'-3')	Amplicon length (bp)	Supplementary Reference
ATP5B	fwd : CAGCATTTGGGTGAGAGCAC rev : TCTGCCCAAAGTCTCAGGAC	129	(S1)
RIG-I	fwd : GTTGTCCCCATGCTGTTCTT rev : GCAAGTCTTACATGGCAGCA	124	(S2)
IFN- β	fwd : CTCTCCTGTTGTGCTTCTCC rev : GTCAAAGTTCATCCTGTCCTTG	152	(S3)
IL-6	fwd : AGCCACTCACCTCTTCAGAAC rev : GCCTCTTTGCTGCTTTCACAC	119	(S4)
EGFP ORF top	fwd : CCTGAAGTTCATCTGCACCAC rev : GCATGGCGGACTTGAAGAAG	137	this paper
EGFP ORF middle	fwd : GACGACGGCAACTACAAGAC rev : GTTGTACTCCAGCTTGTGCCC	129	this paper
EGFP ORF~3'UTR (miR-206)	fwd : TGCATCTCGAGTGATAGCCAC rev : CAAAGACCAAGAGGTACAGGTG	114	this paper
EGFP ORF~3'UTR (miR-302a-5p)	fwd : GCATCTCGAGTGATAGAGCAAG rev : CAAAGACCAAGAGGTACAGGTG	114	this paper

Supplementary Figures

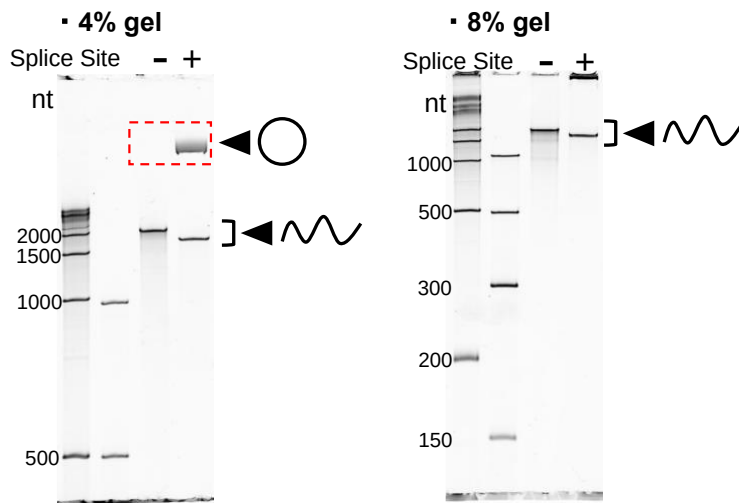


Figure S1. Denaturing polyacrylamide gel images for RNAs after the gel purification. DynaMarker RNA High (BioDynamics Laboratory) and Low Range ssRNA Ladder (NEB) were used as molecular weight markers. circRNA band is shown by red dotted rectangle. While only a minor contamination of nicked circRNA product was seen, which was also observed in the previous reports (3, 16). The gel images are representative data from at least two independent experiments.

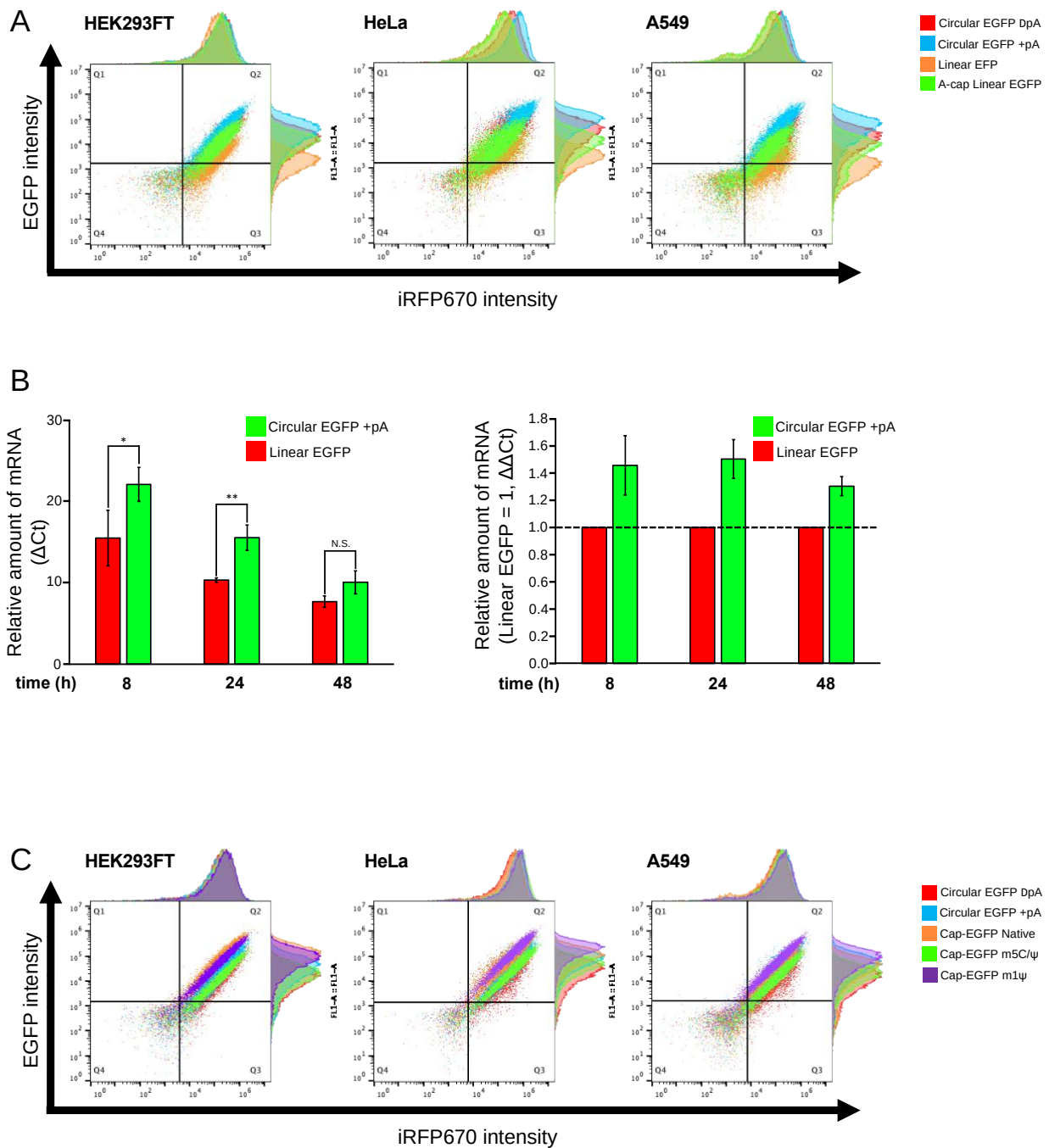


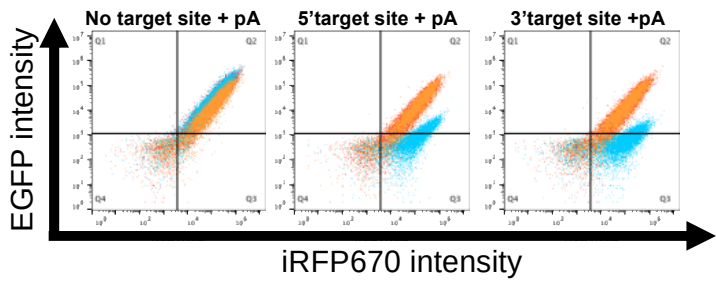
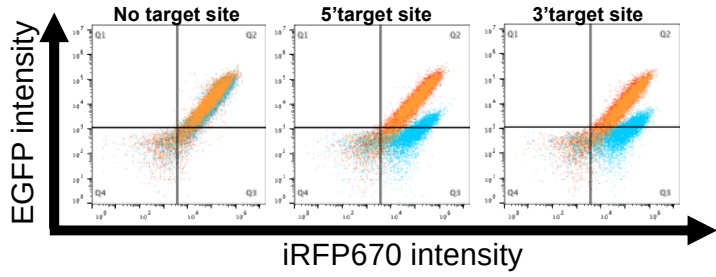
Figure S2. (A) Scatter plots generated from flow cytometry analysis in Figure 3B. (B) Analysis of the relative amount of circRNA and linRNA by RT-qPCR. The residual RNA level was compared between Linear EGFP and Circular EGFP +pA transfection after 8, 24 and 48 h in HEK293FT cells. Target mRNA quantities were normalized by ATP5B mRNA. Levels of significance are denoted as $*P < 0.05$, $**P < 0.01$ (two-tailed unpaired Student's or Welch's *t*-test determined by *F*-test). N.S. means non-significant ($P > 0.05$).

(C) Scatter plots generated from flow cytometry analysis in Figure 3C. All data in this figure are presented as mean $\pm$ SD, $n = 3$. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670.

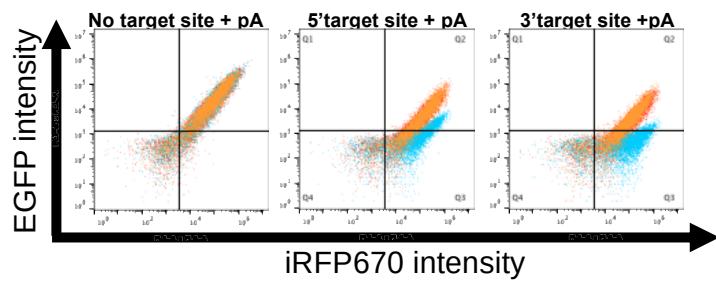
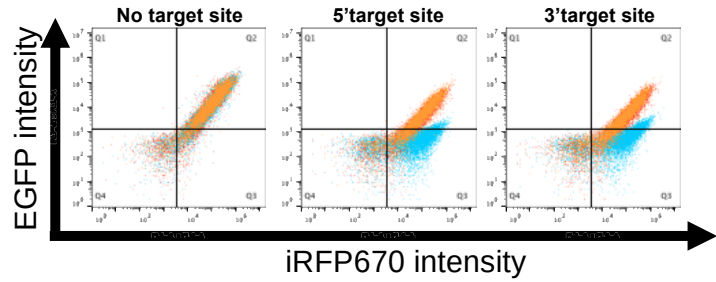
A

■ mimic -
■ mimic +
■ Negative Control mimic +

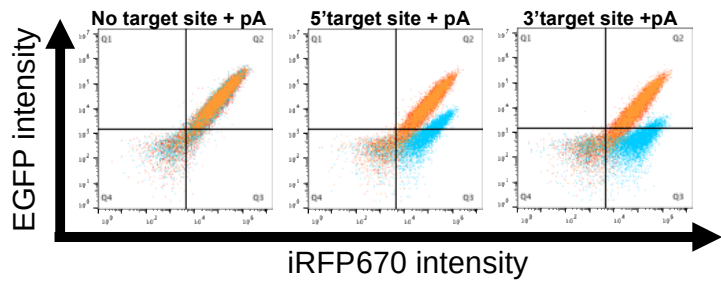
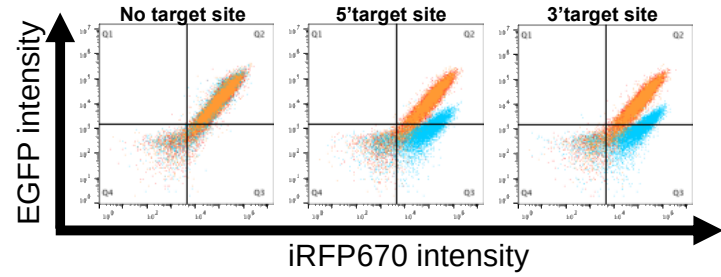
hsa-miR-206 responsive



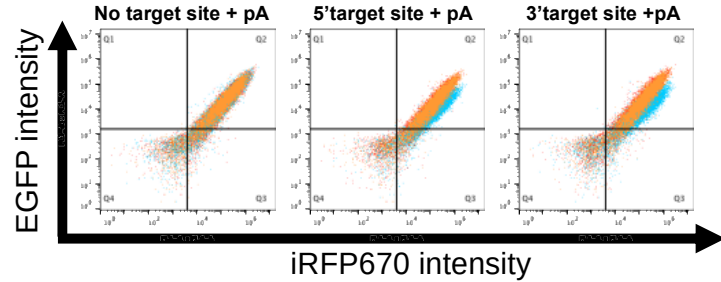
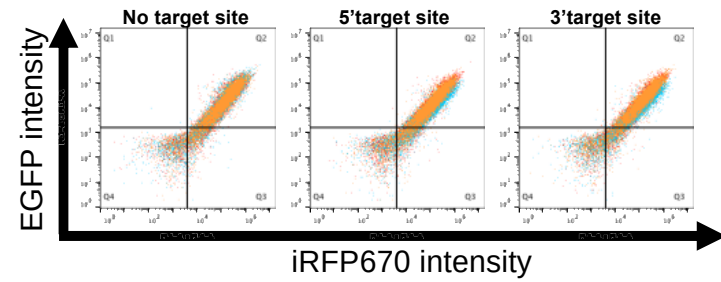
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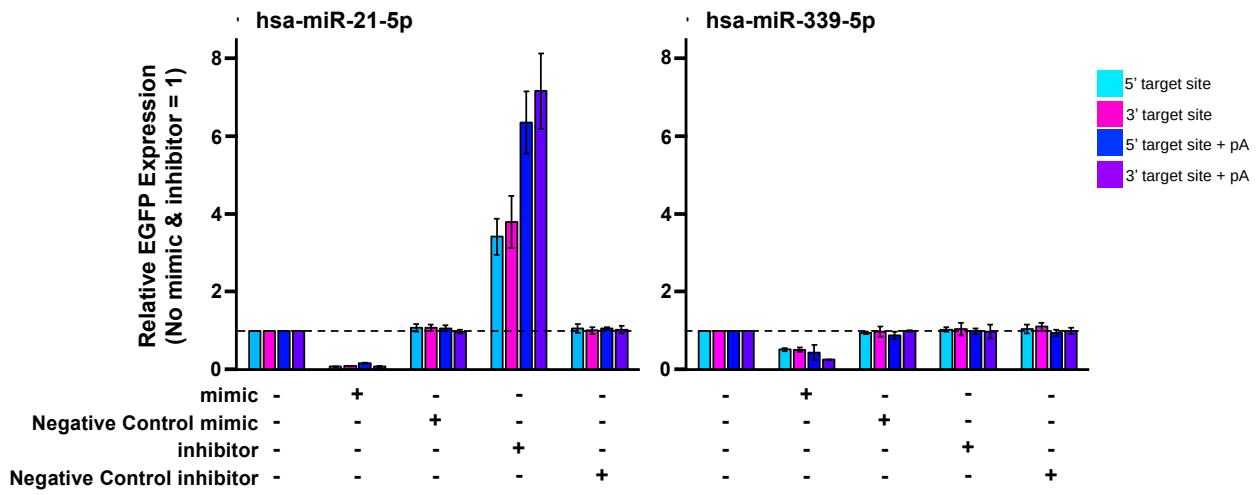
hsa-miR-302a-5p responsive



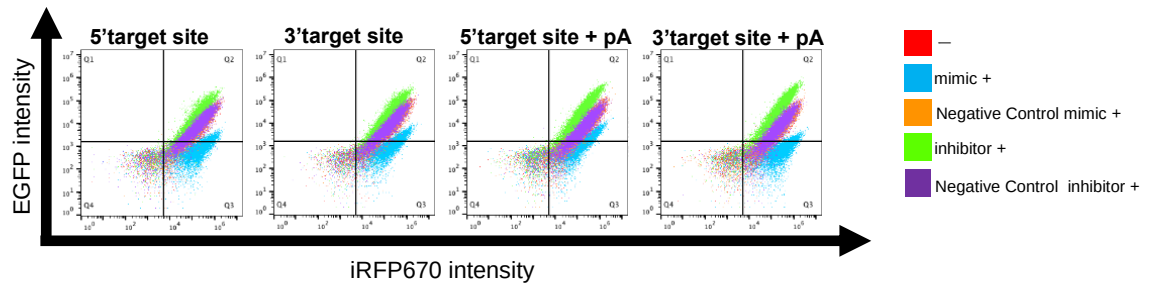
hsa-miR-339-5p responsive



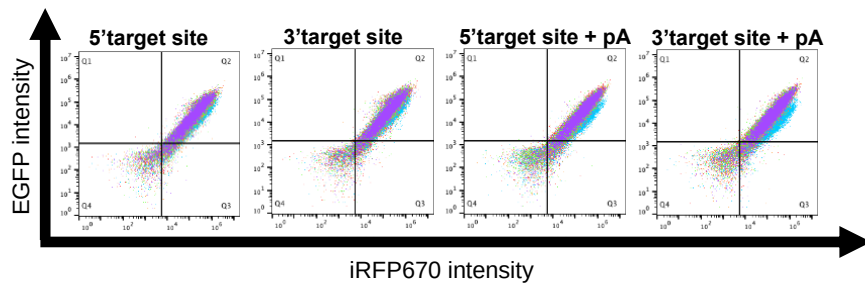
B



hsa-miR-21-5p responsive + mimic or inhibitor



hsa-miR-339-5p responsive + mimic or inhibitor



C

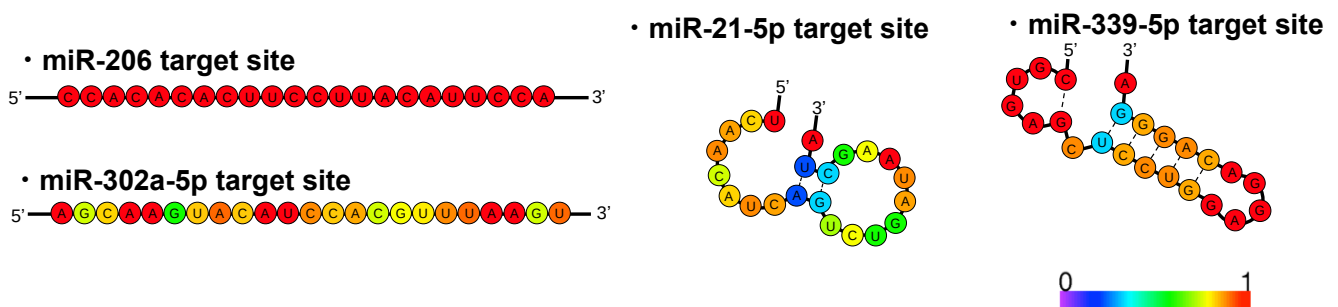


Figure S3. (A) Scatter plots generated from flow cytometry analysis in Figure 4A and 4B. (B) Evaluation of miR-21-5p- or miR-339-5p-responsive circRNA switches with co-transfecting miRNA mimic or inhibitor. (C) Secondary structure predictions of miRNA target sites used in Figure 4A and 4B with the base-pairing probabilities shown in color. Base-pairing probabilities were calculated by CentroidFold software (S5). The red pairs have a high probability of forming, green pairs have a medium probability of forming, and blue pairs have a low probability of forming. The normalized scale showing zero to one probability is shown on the lower right. All data in this figure are presented as mean $\pm$ SD, $n = 3$. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670.

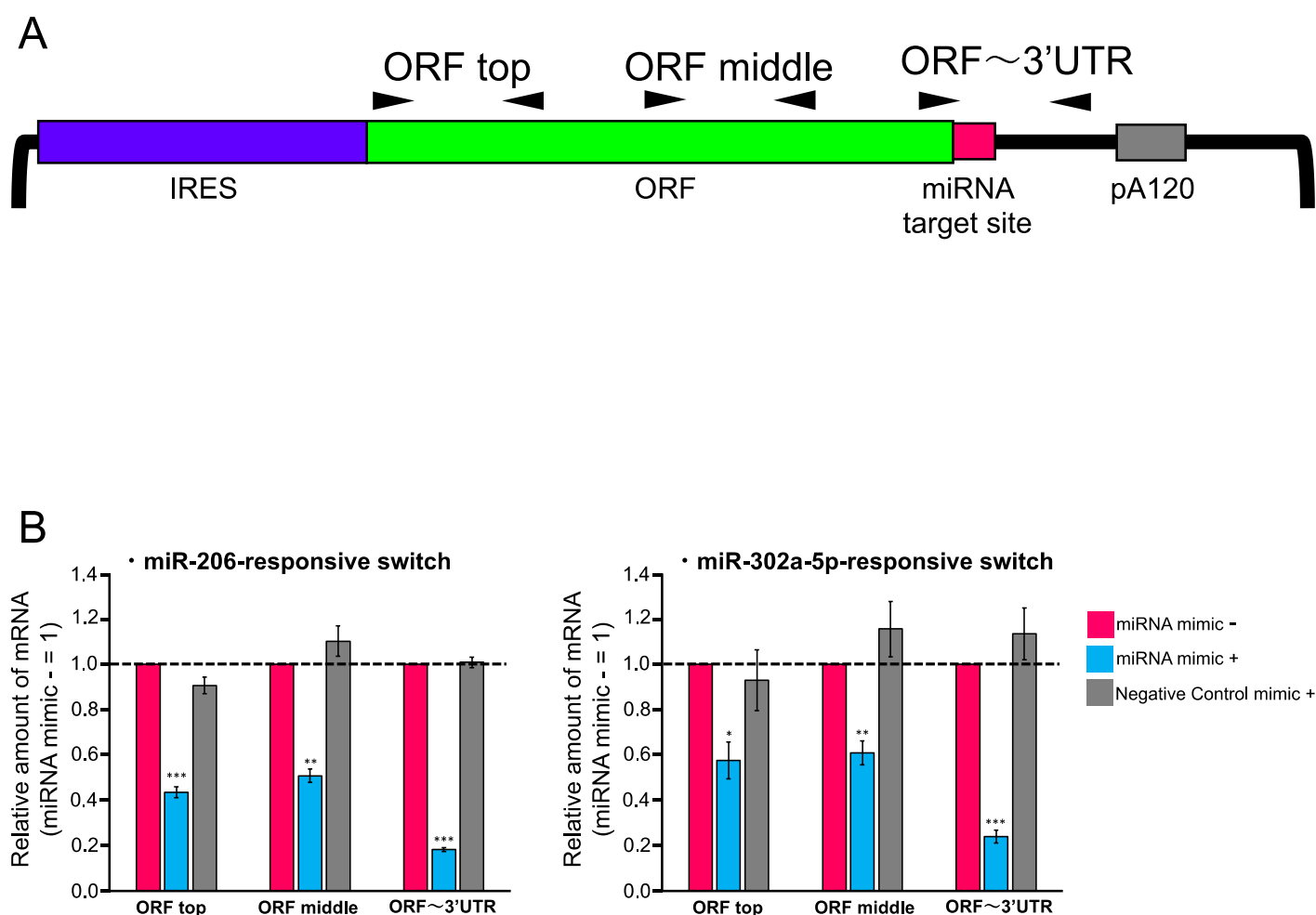


Figure S4. (A) Schematic illustrations of primer pairs on miRNA-responsive circRNA switches. Three different regions were amplified by each primer pair. (B) Evaluation of mRNA amount by RT-qPCR after 24 h of transfection in HEK293FT cells. The relative mRNA amount was calculated by normalizing the sample without a miRNA mimic (magenta). Target mRNA quantities were normalized by ATP5B mRNA. Levels of significance are denoted as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (two-tailed unpaired Student's or Welch's *t*-test determined by *F*-test, mimic + vs Negative Control mimic +). N.S. means non-significant ($P > 0.05$). All data in this figure are presented as mean $\pm$ SD, $n = 3$.

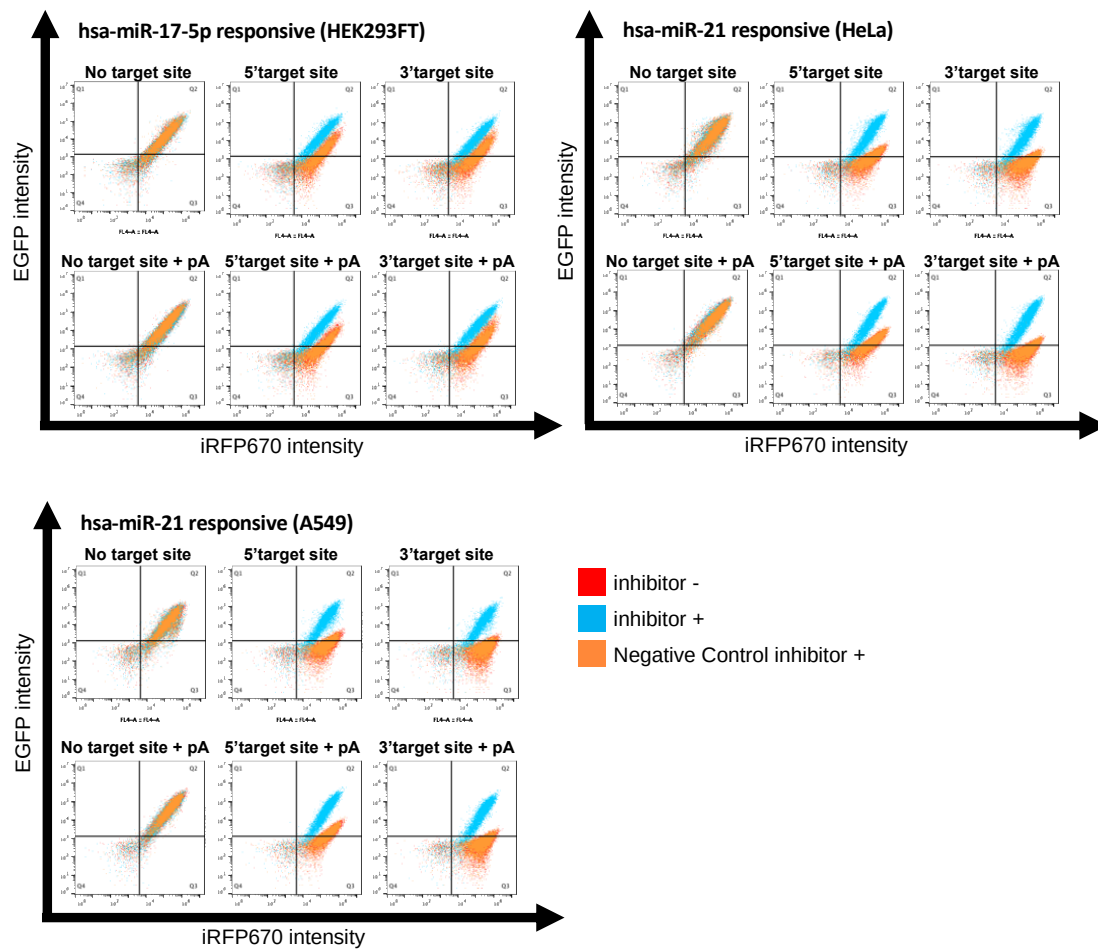


Figure S5. Scatter plots generated from flow cytometry analysis in Figure 4C, 4D. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670.

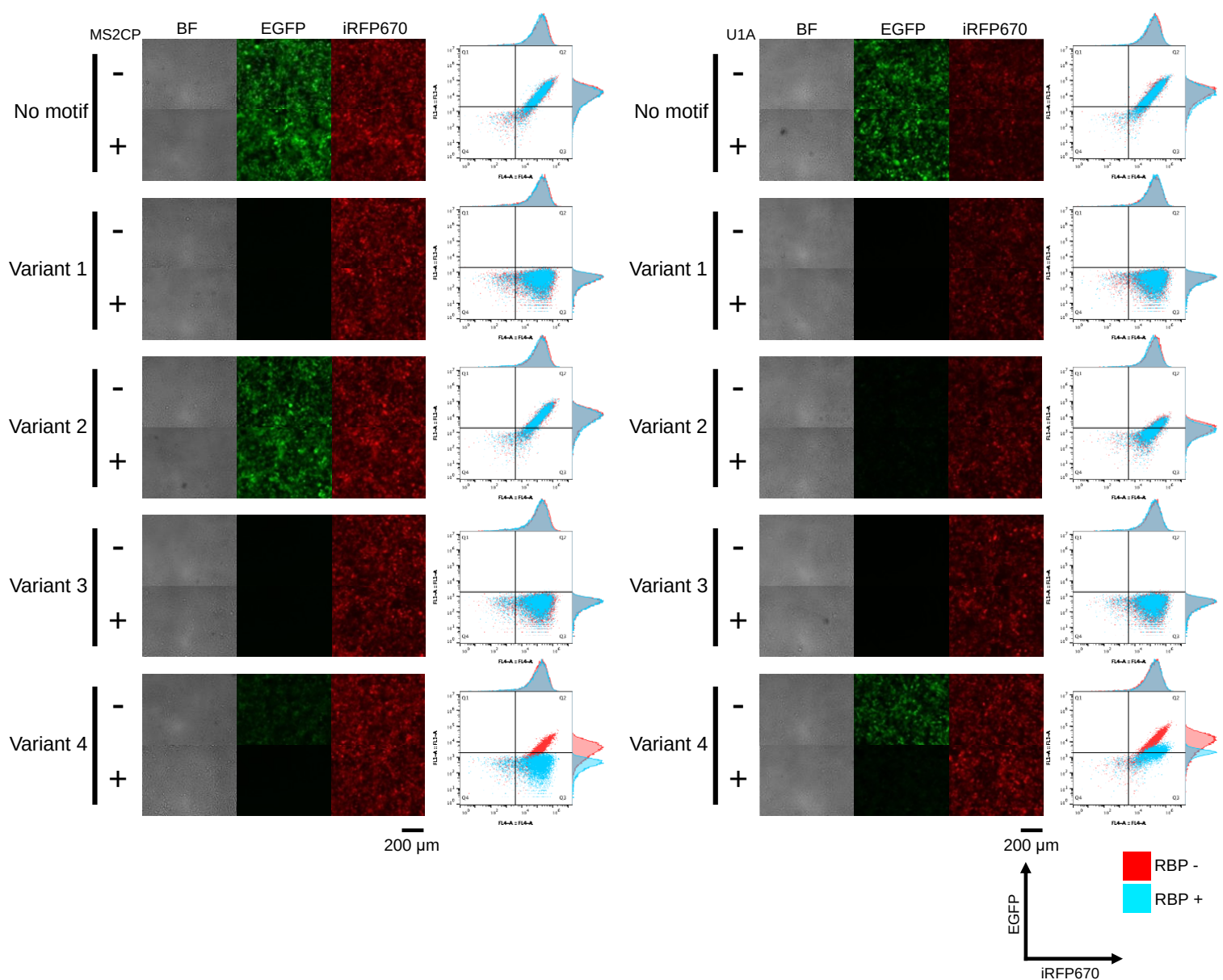


Figure S6. Fluorescent microscopy images and scatter plots from flow cytometry analysis in Figure 5B (including variants 1-4). The scale bar in fluorescent images indicates 200 µm. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670.

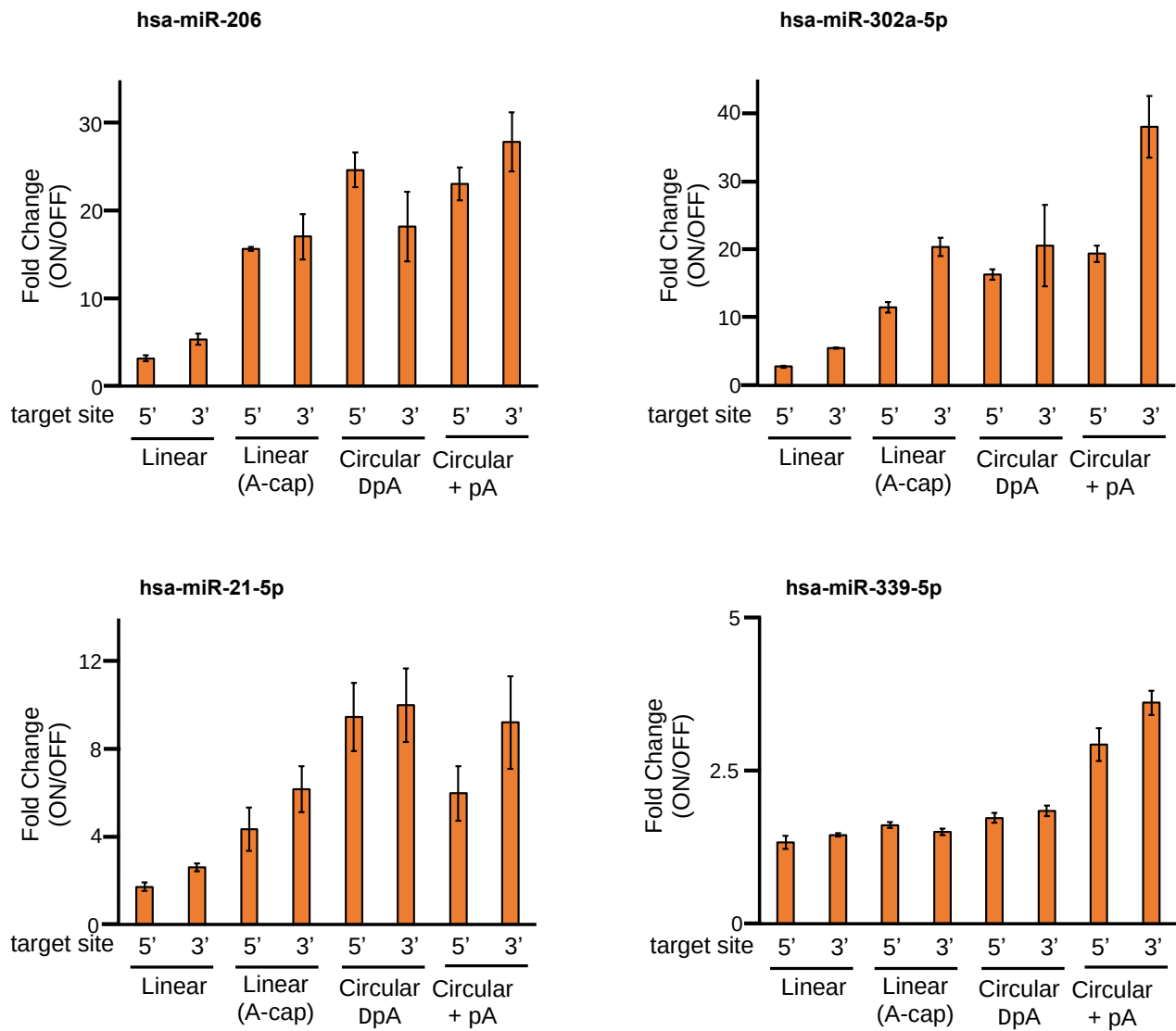
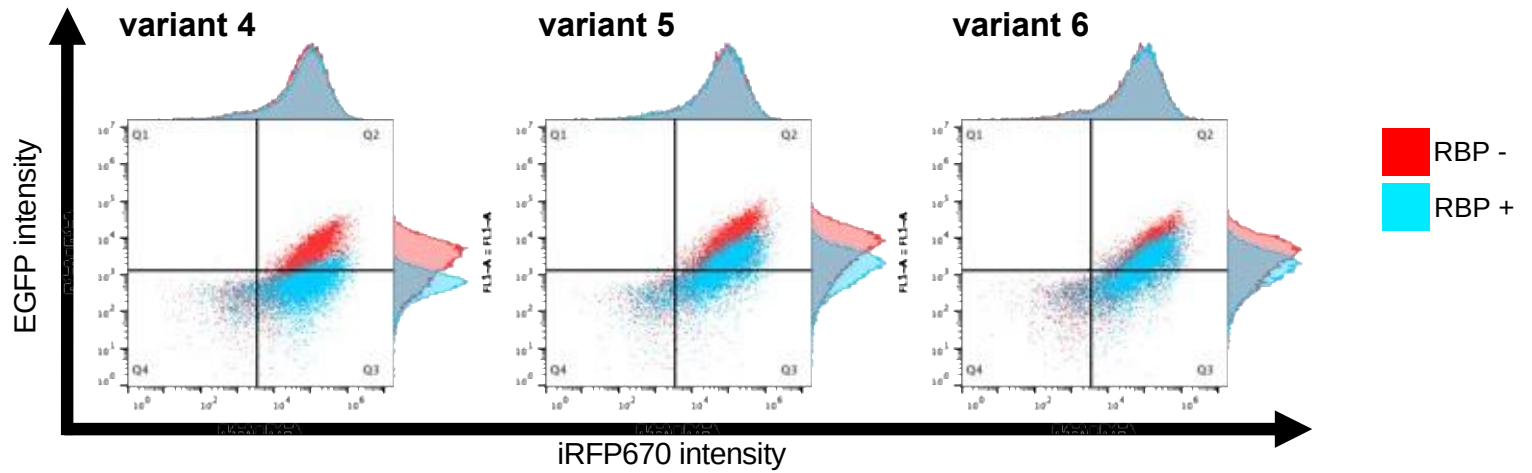


Figure S7. Comparison of miRNA-responsive circRNAs and linear mRNAs with the same sequence components of circRNAs in HEK293FT cells. For circRNA constructs, the results from a single comparison performed simultaneously were presented in two separate graphs, Figure 4B and S7. All data in this figure are presented as mean $\pm$ SD, $n = 3$.

MS2CP responsive variants



U1A responsive variants

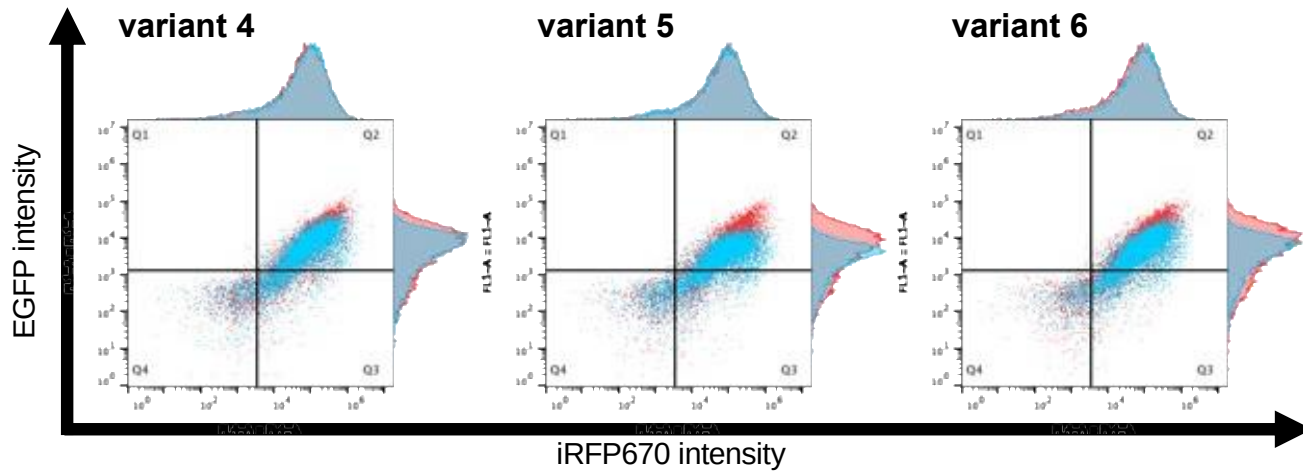


Figure S8. Scatter plots generated from flow cytometry analysis in Figure 5C. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670.

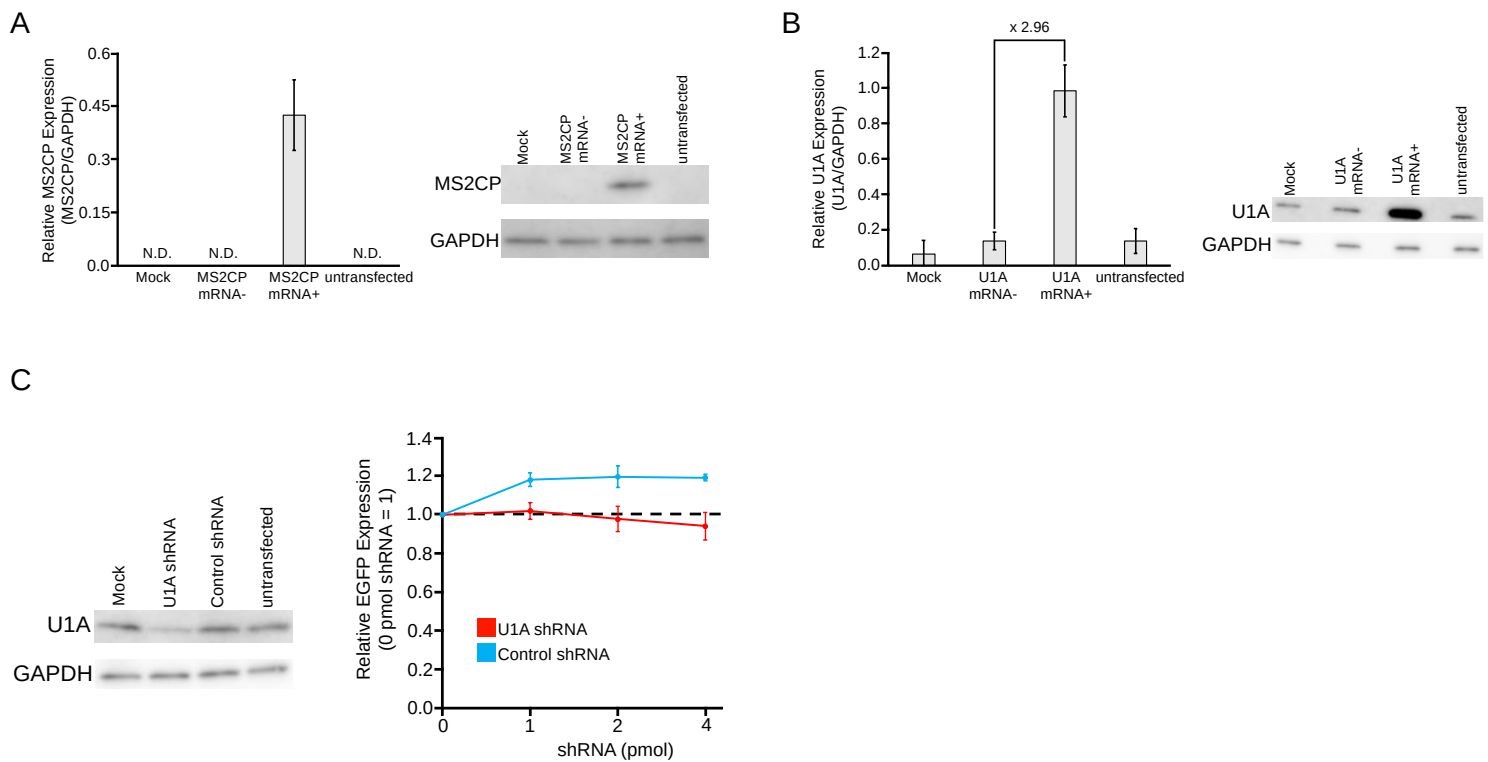
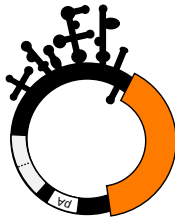


Figure S9. (A) Western blot analysis of MS2CP. 0.05 pmol of *MS2CP*-coding mRNA, 0.3 pmol of each reporter mRNA and transfection control mRNA were co-transfected. Cell culture was performed in 24-well format. (B) Western blot analysis of U1A. 0.15 pmol of *U1A*-coding mRNA, 0.3 pmol of each reporter mRNA and transfection control mRNA were co-transfected. Cell culture was performed in 24-well format. (C) Western blot analysis of U1A (left) and EGFP reporter expression from U1A-responsive circRNA switch (right) with shRNAs. 4 pmol of shRNAs were co-transfected for knockdown evaluation in western blotting. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected and 1, 2 or 4 pmol of shRNA was co-transfected. The slight increase in reporter expression upon co-transfection of control shRNA has also been observed in previous studies (S6). All data in this figure are presented as mean $\pm$ SD, $n = 3$. The experiments were performed in HEK293FT. GAPDH was used as a loading control for all western blot analyses. The band images of western blotting shown are representative of data from three biological replicates. The contrast of each western blot band shown in the figures was adjusted (Figure S9A • B right, S9C left). Quantification of band intensity was performed using image data before contrast adjustment (Figure S9A • B left graph).

A

Circular MetLuc2 +pA

Cap-MetLuc2
(m5C/ψ, m1ψ)

B

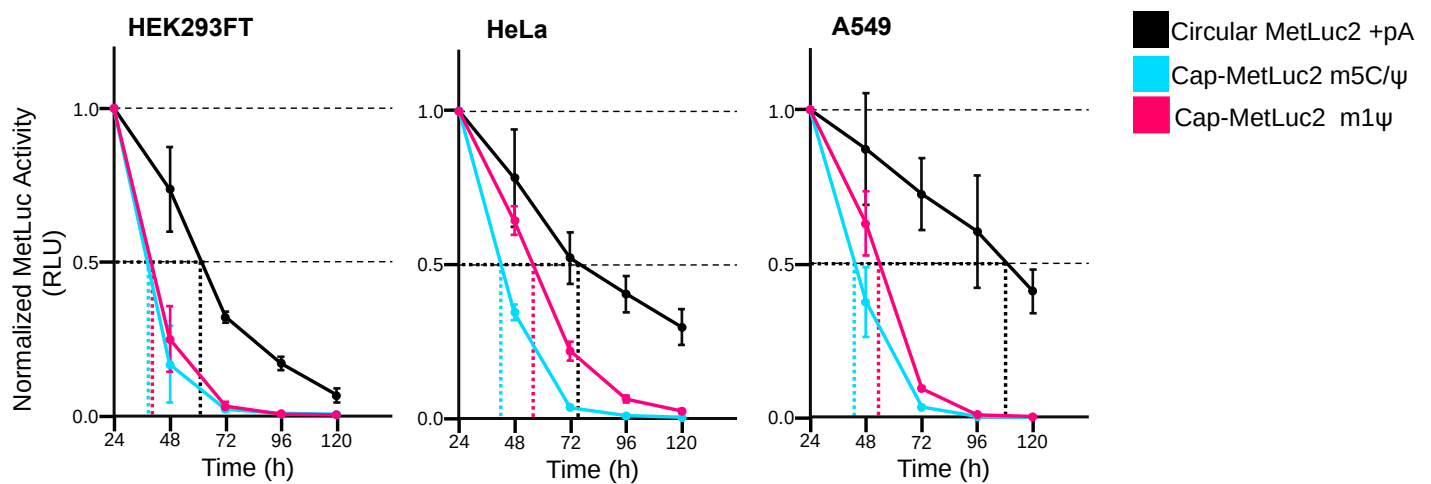


Figure S10. (A) Structure illustration of mRNAs used in the secreted luciferase assay. All mRNAs code Metridia Luciferase (*MetLuc2*) as a reporter gene. (B) Comparison of circRNAs and conventional linear mRNA with base substitutions (m5C/ψ, m1ψ). 45 fmol of reporter mRNA were transfected, and cell culture was performed in 24-well format. All data in this figure are presented as mean $\pm$ SD, $n = 3$.

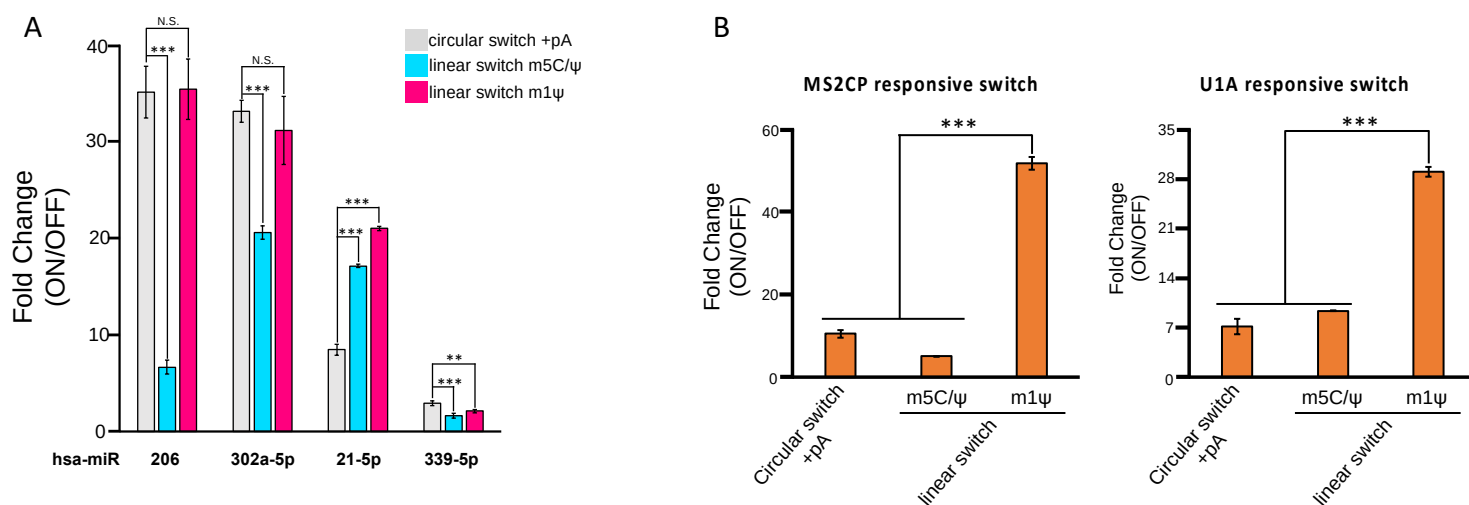


Figure S11. (A) Comparison of the designed miRNA-responsive circRNAs and linear mRNAs with base substitution (m5C/ψ, m1ψ) in HEK293FT cells. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected, and cell culture was performed in 24-well format. 0.25 pmol of specific miRNA mimic or Negative Control mimic was co-transfected for evaluation. (B) Comparison of MS2CP- or U1A-responsive circRNAs and linear mRNAs with base substitution (m5C/ψ, m1ψ) in HEK293FT cells. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected and cell culture was performed in 24-well format. 0.05 pmol of *MS2CP*-coding mRNA or 0.15 pmol of *U1A*-coding mRNA was co-transfected for evaluation. Levels of significance are denoted as * $P < 0.05$, *** $P < 0.001$ (Dunnett's test). N.S. means non-significant ($P > 0.05$). All data in this figure are presented as mean $\pm$ SD, $n = 3$.

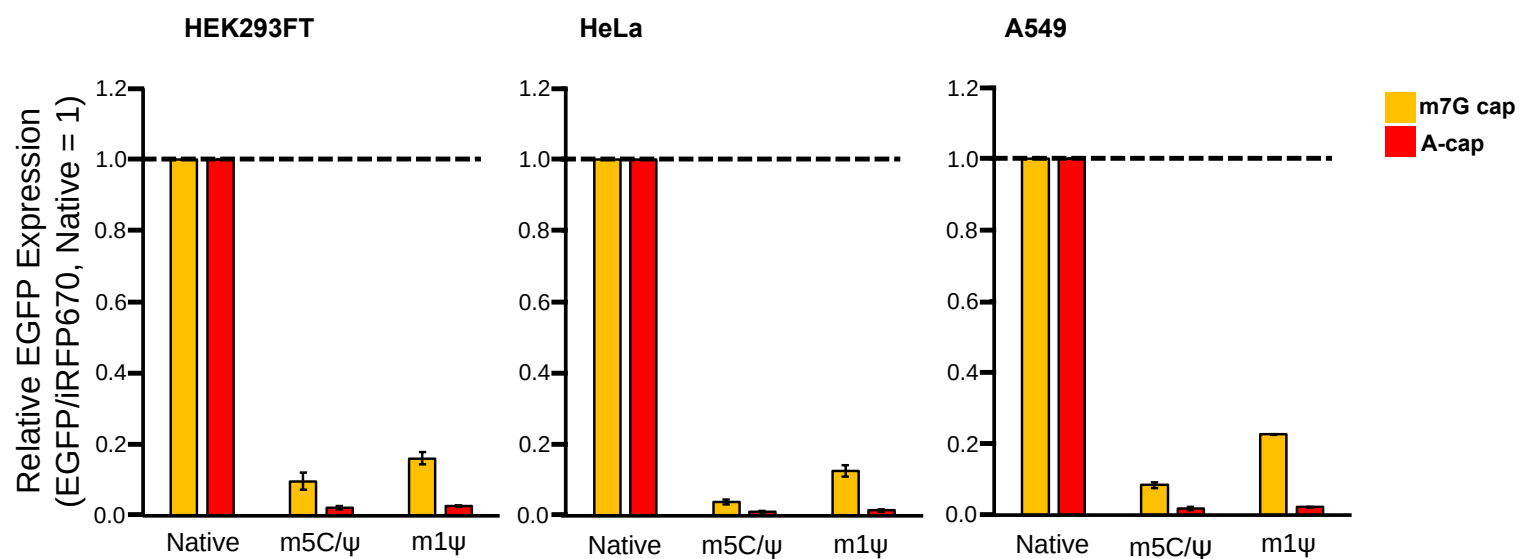


Figure S12. Translatability of linear mRNAs (m7Gcap-EGFP and A-cap-EGFP) with IRES transcribed with non-modified bases (Native) or modified bases (m5C/ψ, m1ψ). 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected, and cell culture was performed in 24-well format. All data in this figure are presented as mean $\pm$ SD, n = 3.

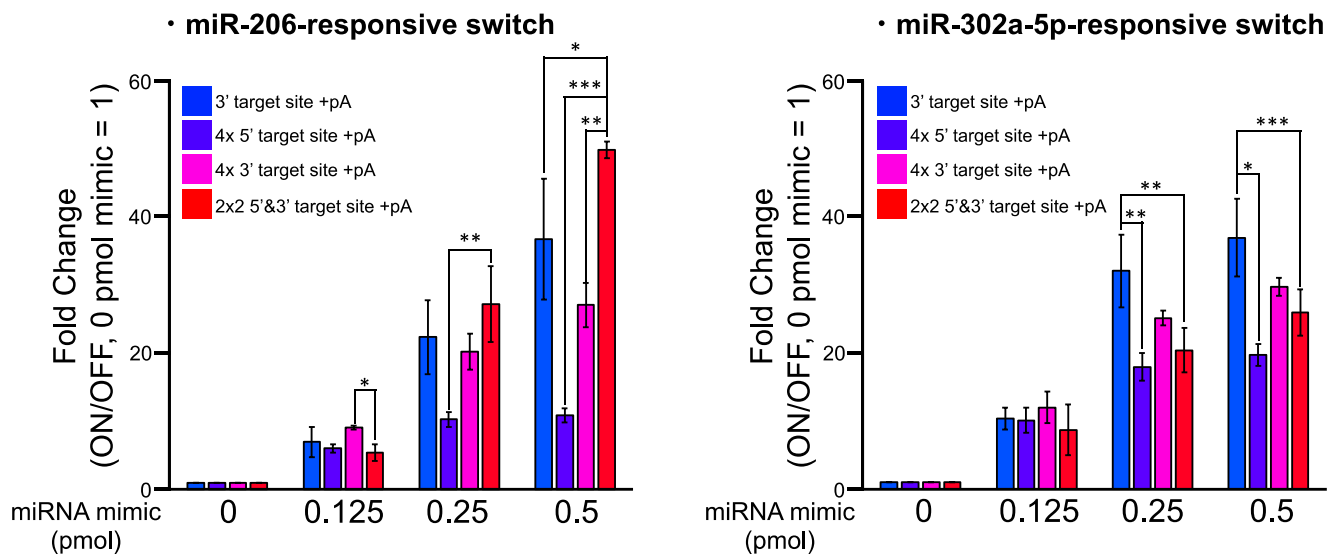


Figure S13. Evaluation of miR-206 or miR-302a-5p-responsive circRNA switches with multiple copies and its patterns of the miRNA target site. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected and cell culture was performed in 24-well format. 0.125, 0.25, 0.5 pmol of specific miRNA mimic was co-transfected for evaluation. Levels of significance are denoted as * $P < 0.05$, *** $P < 0.001$ (Dunnett's test). N.S. (non-significant, $P > 0.05$) pairs were not denoted on the graph. All data in this figure are presented as mean $\pm$ SD, $n = 3$.

Supplementary Sequences

Supplementary Sequences. mRNA sequences used in this report

Circular EGFP Δ pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGU**UGA**
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
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GAAUGCGGCUAUACCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUCAUUUUUAU
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gcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucuccuugcac
cuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGAC

GCU**ACGGACU**UAAAUAUUGAGCCUUAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAA
CUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUA
AUUAGUAAGACCAGUGGACAAUCGACGGAUACAGCAUAUCUAG

Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAAACGGUCGUGUGGGU**U**CAAGUCCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGU**U**GAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUACCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU**CGUUACCAU**AUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAU**AUCCCUU**GUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAU**UCAU**UGUUAAGUUGAAUACAGCAA**au**ggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccugaguucac
ugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacuucagaaggaggacggcaauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucagaucgcca
caaucaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagaccccaacgagaagcgcgaucau
gguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucauau
gcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucucccuugcac
cuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA

AAA
AAAGGCUAUUAUGCGUUAACGGCG
AGACGCU**ACGGACU**UAAAUAUUGAGCCUUAAGAAGAAAUUCUUAAGUGGAUGCUCU
CAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGU
AGUAAUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAAC
AGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCC
CGGGUACCGAGCUCGAAUU

Circular EGFP Δ pA Δ IRES (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
augggauccgugagcaagggcgaggagcuguuacccgggugggugcccauccuggucgagcuggacggcgac
guaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcugaccugaagu
ucaucugcaccaccggcaagcugcccugcccugggccaccucgugaccaccucgaccuacggcgugcagug
cuucagccgcuaccccgaccacaugaagcagcagcagcuucuuaaguccgccaugcccgaaggcuacguccag
gagcgcaccaucucuuaaggacgacggcaacuacaagaccgcgcccaggugagauguucgagggcgacacc
uggugaaccgcaucgagcugaagggcaucgacuuaaggaggacggcaacaucggggcacaagcuggagu
acaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuuaagau
ccgccacaacucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggcc
ccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaagaccccaacgagaagcgga
ucacaugguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagauc
ucauauagcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucucc
uugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUAACGGC
GAGACGCUACGGACU**UAAAUAUUGAGCCUUAAGAAGAAAUUCUUAAGUGGAUGCUC**

UCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAG
UAGUAAUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

Circular EGFP +pA Δ IRES (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA

CCUUAACGGUCGUGUGGGUUCAAGUCCCUCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
augggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgac
guaaacggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccugaagu
ucaucugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccucgaccuacggcgugcagug
cuucagccgcuacccccgaccacaugaagcagcagacuucuuaaguccgccauGCCgaaggcuacguccag
gagcgcaccauucuuaaggagcagggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccc
uggugaaccgcaucgagcugaagggcaucgacuuaaggaggacggcaacaucggggcacaagcuggagu
acaacuacaacagccacaacgucuauaauauggccgacaagcagaagaacggcaucaaggugaacuuaagau
ccgccacaacaucgaggacggcagcgugcagcugcccgaccacuaccagcagaacacccccaucggcgacggcg
ccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaagaccccaacgagaagcgga
ucacaugguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagauc
ucauauugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucuccc
uugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAA
AAA
AAAGGCUAUUAUGCGUAC
CGGCGAGACGCUACGGACUUAUUAAUUGAGCCUUAAGAAGAAAUUCUUUAAGUGGAU
GCUCUCAAAACUCAGGGAACCUAAUUCUAGUUUAUAGACAAGGCAAUCCUGAGCCAAGCC
GAAGUAGUAAUUAAGUAGAACAGCAGUGGACAAUCGACGGAUAAACAGCAUAUCUAGACACAG
GAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGA
UCCCCGGGUACCGAGCUCGAAUU

Linear EGFP

Group I intron, CVB3 IRES, EGFP

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAUAAGACAC
CGGUCGCCACC UAAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUACCGCAUUC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUU

AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU CGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
AGCUUGAAAGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAA **augggaucc**
gugagcaagggcgaggagcuguuacccgggguggugcccuccugugcagcuggacggcgacguaaacggccacaag
uucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugacccugaaguucaucugcaccaccggcaagc
ugcccuguccugcccaccucgugaccaccugaccuacggcgugcagugcuucagccguaccccagaccacaugaa
gcagcacgacuucucaaguccgccaugcccgaaggcuacguccaggagcgacccaucuucucaaggacgacggcaac
uacaagaccgcgcccagggugaaguucgagggcgacaccucggugaaccgcaucgagcugaaggggcaucgacucaag
gaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcag
aagaacgggcaucaaggugaacucaagaauccgccacaauccgaggacggcagcgugcagcucgccgaccacuaccagc
agaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaga
ccccaacgagaagcgcgaucaauguccugcuggaguucgugaccgcgcccgggaucacucucggcauggacgagcug
uacaagagaucucauauugcaucucgagugauag ucuagaccuucugcggggcuugccuucuggccaugcccuucuuc
cuccuugcaccguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUAACCG
CGAGACGCU **ACGGACUU**AA
AA
AAAAAAAAAAAA

Cap-EGFP

EGFP

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAUAGACACCGGU **cgccacc** **auggga**
uccgugagcaagggcgaggagcuguuacccgggguggugcccuccugugcagcuggacggcgacguaaacggccac
aaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugacccugaaguucaucugcaccaccggc
aagcugcccuguccugcccaccucgugaccaccugaccuacggcgugcagugcuucagccguaccccagaccacu
gaagcagcacgacuucucaaguccgccaugcccgaaggcuacguccaggagcgacccaucuucucaaggacgacggc
aacuacaagaccgcgcccagggugaaguucgagggcgacaccucggugaaccgcaucgagcugaaggggcaucgacuuc
aaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaag
cagaagaacggcaucaaggugaacucaagaauccgccacaauccgaggacggcagcgugcagcucgccgaccacuacc
agcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaa
agacccaacgagaagcgcgaucaauguccugcuggaguucgugaccgcgcccgggaucacucucggcauggacga
gcuguacaagagaucucauauugcaucucgagugauag ucuagaccuucugcggggcuugccuucuggccaugcccuuc
uucucuccuugcaccguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAA
AA
AAAAAAAAAAAA

Cap-iRFP670

iRFP670

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcgccacc**ATGgc**
gcuagggucgaucucaccuccugcgauccgagccgauccacauccccggcagcauucagccgugcggcugccugcua
gccugcgacgcgcaggcggugcggaucacgcgcauuacggaaaugccggcgcuucuuuggacgcgaaacuccgcgg
gucggugagcuacucgccgauuacuucggcgagaccgaagcccaugcgucgcaacgcacugggcgaguccuccgauc
caaagcgaccggcgcuauucggguuggcgagccgacggccgacccuucgacauucacugcaucgccauga
cgguacaucgaucaucgaguucgagccugcgggccgaacaggccgacaaucgcugggcugacgcggcagaucauc
gcgcgaccaaagaacugaagucgcucgaagagauggccgcagggugccgcgcuauucgaggcgauucggcuau
caccgcgugauguuacgcgucggagcgccgucgggauggugaucggcgaggcgaaagcgagcgaccucgag
agcuuucucggucagcacuuuccggcgucgugucccgagcaggcgccgcuacugacuugaagaacgcgauccgcg
uggucucggauucgcgcggcaucagcagccggaucgugcccagcagcagccuccggcgccgcgcucaucgucguu
cgcgaccugcgagcaucucgcccugccaucucgaauuucugcggaacauggggcgucagcgccucgaugucgucg
aucaucauugacggcacgcuauggggauugaucaucugucaucauuacgagccgcgugccgugccgauggcgagcgc
gucgcgccgaauguucgccgacuucuuacgcgucacuucaccgcgcccaccaccaacgcagaucauau**GCAU**
CUCGAGUGAUAGucuagaccuucugcggggcuugccuucugggcaugcccuucucucccuugcaccuguacc
ucuuggucuuuGAAUAAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AAAAAAAAAAAAAAAAAAAAAAAAAAAA

Circular MetLuc2 +pA (**bold: circularized**)

Group I intron, CVB3 IRES, MetLuc2

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUAACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAAUCUGCGGAGCACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAU

UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU CGUUACCAUUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA **augga**
caucaaggugguguucaccucgguguucagcgccugugcaggccaagagcaccgaguucgaccccaacauc
gacaucguggggccuggaaggcaaguucggcaucaccaaccuggaaccgaccguucaccaucugggagacca
uggaagugaugaucaaggccgacaucgacacccgacggggccagcaacuucguggccaccgagaccgacgc
caaccggggcaagaugcccggcaagaagcugccccugggccgucaucauggaaauggaagccaacgccuuaag
gcccggcugcaccggggcugccugaucugccugagcaagaucagugcaccgccaagaugaagguguacauc
ccggcaggugccacgacuacggcggcgacaagaaaaccggccaggccggcaucguggggcgccaucugggacau
ccccgagaucagcgguucaagaaauggccccauggaacaguucaucgcccagguggacagaugcgccagc
ugcaccaccggcugccugaaggggccuggccaacgugaagugcagcgagcugcugaagaaguggcugcccgacc
gcugcgccagcuucgcccgacaagauccagaaagguggcacaacaucaggggcauggccggcgacaggugauc
uagaccuucugcggggcuugccuucuggccaugcccuucucucucccuugcaccuguaccucuuggucuuu
GAAUAAAGCCUGAGUAGGAAA
AA
AAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCU **ACGGACUU**
AAUAAUUGAGCCUUAAGAAGAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAAC
CUAAAUUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAAGUAGAC
CAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAU
UACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCG
AAUU

Cap-MetLuc2

MetLuc2

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAUAGACACCGGU **cgccacc** **auggac**
aucaaggugguguucaccucgguguucagcgccugugcaggccaagagcaccgaguucgaccccaacaucgacauc
guggggccuggaaggcaaguucggcaucaccaaccuggaaccgaccguucaccaucugggagaccauggaagugaug
aucaaggccgacaucgcccacacccgacggggccagcaacuucguggccaccgagaccgacgccaaccggggcaagaugc
ccggcaagaagcugccccugggccgucaucauggaaauggaagccaacgccuuaaggccggcugcaccggggcugccu
gaucugccugagcaagaucagugcaccgccaagaugaagguguacaucucccgaggugccacgacuacggcgga
caagaaaaccggccaggccggcaucguggggcgccaucguggacauccccgagaucagcgguucaagaaauggcccc
auggaacaguucaucgcccagguggacagaugcgccagcugcaccaccggcugccugaaggggccuggccaacgugaag
ugcagcgagcugcugaagaaguggcugcccgaccgugcgccagcuucgcccgacaagauccagaaagguggcacaac
aucaagggcauggccggcgacaggugaucuaagaccuucugcggggcuugccuucuggccaugcccuucucucucccu
gcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA

AAAAAAAAAAAAAAAAAAAAAAAAAAAA

5'T206 Circular EGFP ΔpA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-206 target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCCCACAC****
****ACUUCUUAACAUUC****CA****GCCACC**UUAACAGCCUGUGGGUUGAUCCACCCACAGGCC**
CAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCC
UCCCCAACUGUAACUUAAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCA
GCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCAC
GCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAAC
ACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAU
GAGUCACCGCAUUCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCA
UGGGGAAACCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUA
GUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAUCCUAAACUGCGGAGCACACACCCU
CAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUG
GGUGUCCGUGUUUCAUUUUUAUCCUUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU
CGUUAACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCC
CUUUGUUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAACAUUACAAUUAUUGUU
AAGUUGAAUACAGCAAA**augggauccgugagcaagggcgaggagcuguucaccgggguggugcccauc**
cuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccacc
uacggcaagcugaccugaagucaucugcaccaccggcaagcugcccugcccuggcccaccucgugacca
cccugaccuacggcgugcagugcuucagccguaccccgaccacaugaagcagcagcagcuucuaaguccgc
caugcccgaaggcuacguccaggagcgcaccaucuuucaaggacgacggcaacuacaagacccgcgcccag
gugaaguucgagggcgacaccuggugaaccgcaucgagcugaagggcaucgacuuaaggaggacggcaaca
uccuggggcaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacgg
caucaaggugaacuuaagaucggccacaacaucgaggacggcagcgugcagcucggcaccacuaccagcag
aacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaa
agacccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucggcaug
gacgagcuguacaagagaucucauugcaucucgagugauag**ucuagaccuucugcggggcuugccuucugg**
ccaugcccuucucucuccuugcaccuguaccucuuggucuuu**GAAUAAAGCCUGAGUAGGGGCU**
AUUAUGCGUUAACGGCGAGACGCU**ACGGACUUA****AAUAAUUGAGCCUUA****AAGAAGAAU**
UCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACC**UAAUUCUAGUUAUAGACAAGGCAAU**

CCUGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAGUGGACAAUCGACGGAUAAACAGCA
UAUCUAG

5'T206 Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-206 target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAAACGGUCGUGUGGGU**UCAAGUCCUCCACCCCCACGCCG**GAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUC**CCACAC**
ACU**UCCUACAU****UCCAGCCACC**UAAAAACAGCCUGUGGGUUGAUCC**ACCCACAGGCC**
CAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCC
UCCCCAACUGUAACUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCA
GCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCAC
GCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAAC
ACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAU
GAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCA
UGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUA
GUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAUCCUAAACUGCGGAGCACACACCCU
CAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUG
GGUGUCCGUGUUUCAUUUUUAUCCUUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU
CGUUACCAUAUAGCUAUUGGAUUGGCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCC
CUUUGUUGGGUUUAUACCACUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUU
AAGUUGAAUACAGCAAA**augggauccgugagcaagggcgaggagcuguucaccgggguggugcccauc**
cuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauGCCacc
uacggcaagcugaccugagaaguucaucugcaccaccggcaagcugcccugcccuggcccaccucgugacca
cccugaccuacggcgugcagugcuucagccgcuaaccccgaccacaugaagcagcagcguucuucaaguccgc
caugcccgaaggcuacguccaggagcgacccaucuuucaaggacgacggcaacuacaagacccgcgcgag
gugaaguucgagggcgacaccucggugaaccgcaucgagcugaagggcaucgacuuaaggaggacggcaaca
uccugggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacgg
caucaaggugaacuuaagaucggccacaacaucgaggacggcagcgugcagcucgcccaccacuaccagcag
aacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaa
agacccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucggcaug
gacgagcuguacaagagaucauauugcaucucgagugauagucuagaccuucugcggggcuugccuucugg
ccaugcccuucucucuccuugcaccuguaccucuuggucuuu**GAAUAAAGCCUGAGUAGGAAAA**
AA

AA
GGCUAUUAUGCGUUAACCGGCGAGACGCU**ACGGACUU**AAAUAUUUGAGCCUUAAGAAG
AAAUUCUUUAAGUGGAUGCUCUCAAAACUCAGGGAAACCUAAAUCUAGUUUAUAGACAAGG
CAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAAC
AGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGC
AGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3'T206 Circular EGFP ΔpA (**bold: circularized**)

Group I intron, CVB3 IRES, **EGFP**, miR-206 target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAAGGUCGAUGAGUACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAAUCUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUAACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA**auggg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcugaccugaaguucauc
ugcaccaccggcaagcugcccugcccuggcccaccucugugaccaccucgaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
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acaacagccacaacgucuaaucauggccgacaagcagaagaacggcaucaaggugaacuuaagaucgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagacccaacgagaagcgcgaucau

gguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauag**CACACACUCCUACAUCCA**ucuagaccuucugcggggcuugccuucug
gccaugcccuucucucuccuugcaccuguaccucuuuggucuuuGAAUAAAGCCUGAGUAGGGGC
UAUUAUGCGUUACCGGCGAGACGCU**ACGGACU**UAAAUAUUUGAGCCUUAAGAAGAAA
UUCUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAUUCUAGUUAUAGACAAGGCAA
UCCUGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAGUGGACAAUCGACGGUAACAGC
AUAUCUAG

3'T206 Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, **EGFP**, **miR-206 target site**

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUCAGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUACCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUAACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAA**auagg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcugaccugaagucauc
ugcaccaccggcaagcugcccugcccugggccaccucgugaccaccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacucaagggaggacggcaauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacucaagauccgcca
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cugcugccccgacaaccacuaccugagcaccaguccgcccugagcaaagaccccaacgagaagcgcgaucau
gguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucauau
gcaucucgagugauag**CACACACUCCUACAUCCA**ucuagaccuucugcggggcuugccuucug
gccaugcccuucucucuccuugcaccuguaccucuggucuuuGAAUAAAGCCUGAGUAGGAAA
AA
AA
AGGCUAUUAUGCGUUACCGGCGAGACGCU**ACGGACU**UAAUAAUUGAGCCUUAAGAA
GAAAUUCUUAAGUGGAUGCUCUCAAACUCAGGGAAACC UAAUUCUAGUUAUAGACAAG
GCAAUCCUGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAGUGGACAAUCGACGGAUAA
CAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUG
CAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

4x 5'T206 Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, **EGFP**, miR-206 target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUC**CACAC**
ACUCCUUAACAUUCCACCACACACUCCUUAACAUUCCACCACACACUCCUUAACUUC
CACCACACACUCCUUAACAUUCCA**GCCACC**UAAAAACAGCCUGUGGGUUGAUCCCACC
CACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUU
AUACCCCUCUCCCAACUGUAACUUAAGAAGUAACACACACCGAUCAACAGUCAGCGUG
GCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUAACCCGGACUGAGUAUCAAUAGA
CUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAAC
CUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGUCAGCACUACCCAGUGUAGAUC
AGGUCGAUGAGUCACCGCAUUCUCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGG
CCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUA
UUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAUCCUAACUGCGGAGCA
CACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGA
CUACUUUGGGUGUCCGUGUUUCAUUUUAUCCUUAUACUGGCUGCUUAUGGUGACAAU
UGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCAUCCGGUGACUAAUAGAGCUAU
UAUAUAUCCCUUUGUUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAACAUUACAA
UUAUUGUUAAGUUGAAUACAGCAAA**augggauccgugagcaagggcgaggagcuguuaccggg**
guggugcccauccgugcagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgag
ggcgauccaccuacggcaagcugaccguagaugucaucugcaccaccggcaagcugcccugcccugggccca

cccucgugaccaccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuu
 cuucaaguccgccaugcccgaaggcuacguccaggagcgaccauucuucuaaggacgacggcaacuacaag
 acccgcgccgaggugaaguucgagggcgacaccucggugaaccgcaucgagcugaagggcaucgacuucagg
 aggacggcaacaucgggggacagcuggaguuacaacuacaacagccacaacgucuaaucauaggccgacaa
 gcagaagaacggcaucaaggugaacuucagaucggccacaacaucgaggacggcagcgugcagcucgcccagc
 cacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguc
 cgcccugagcaaaagacccaacgagaagcgcgaucauagguccugcuggaguucgugaccgcccggggau
 acucucggcauggacgagcuguacaagagaucucauagcaucucgagugauagucuagaccuucugcgggg
 cuugccuucuggccaugcccuucucucuccuugcaccuguaccucucuggucuuuGAAUAAAGCCUGA
 GUAGGAAA
 AAA
 AAAAAAAAAAGGCUAUUAUGCGUUAACGGCGAGACGCU**ACGGACUUA**AAUAAUUGAGCC
 UUAAGAAGAAAUUCUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAUUAUAGUUA
 UAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAGUGGACAAUCG
 ACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUG
 CAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

4x 3'T206 Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, **EGFP**, miR-206 target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAACGGUCGUGUGGGUUCAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUAACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU

UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
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 cggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccugaaguucauc
 ugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuuca
 gccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
 caccacuucucaagggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
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 acaacagccacaacgucuaaucauaggccgacaagcagaagaacggcaucaaggugaacuuaagauccgcca
 caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
 cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagacccaacgagaagcgcgaucaacu
 gguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucucauau
 gcaucucgagugauag**CACACACUUCUUAACAUUCCACCACACACUUCUUAACAUUCCAC**
CACACACUUCUUAACAUUCCACCACACACUUCUUAACAUUCCAucuagaccuucugcggggc
 uugccuucuggccaugcccuucucuccuugcaccuguaaccuucuggucuuuGAAUAAAGCCUGA
 GUAGGAA
 AA
 AAAAAAAAAAGGCUAUUAUGCGUUAACGGCGAGACGCU**ACGGACU**UAAUAAUUGAGCC
 UUAAGAAGAAUUCUUAAGUGGAUGCUCUCAAACUCAGGAAACCUAUUAUAGUUA
 UAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAGUGGACAAUCG
 ACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUG
 CAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAU

2x2 5'&3'T206 Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-206 target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUAACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUC**CACAC**
ACUUCUUAACAUUCCACCACACACUUCUUAACAUUCCA**GCCACCUUAAAACAGCCUGU**
GGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUU
UGUGCGCCUGUUUUUAUACCCCUCCCCAACUGUAACUUAAGAAGUAACACACACCGAU
CAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCGGA
CUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCA
ACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACU
ACCCAGUGUAGAUCAGGUCGAUGAGUACCGCAUUCCCACGGGCGACCGUGGCGG

5'T302a-5p Circular EGFP ΔpA (**bold: circularized**)

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGAGCUACCCUAAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGGUUCAAGUCCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACACCGGUCAGCAAG
UACAUCCACGUUUAAGUGCCACC UAAAAACAGCCUGUGGGGUUGAUCCACCCACAGGC

CCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCC
 CUCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACC
 AGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAUAGACUGCUCA
 CGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAA
 CACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGA
 UGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCC
 AUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCU
 AGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAAUGCGGAGCACACACCC
 UCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
 GGGUGUCCGUGUUUCAUUUUUAUCCUAAUACUGGCUGCUUAUGGUGACAAUUGAGAGA
 UCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUC
 CCUUUGUUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAACAUAUACAUAUUGU
 UAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguuacccgggguggugccca
 uccuggugcagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauGCCA
 ccuacggcaagcugaccugaagucaucugcaccaccggcaagcugcccugcccuggcccaccucgugac
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 acauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaaucauggccgacaagcagaaga
 cggcaucaagguagaacuuaagaucggccacaacaucgaggacggcgagcugcagcucgcccgaccacuaccag
 cagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccuga
 gcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgcccgggaucacucucggg
 cauggacgagcuguacaagagaucauauugcaucucgagugauagucuagaccuucugcggggcuugccuu
 cuggccaugcccuucucucuccuugcaccuguaccucuuuggucuuuGAAUAAAGCCUGAGUAGGG
 GCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAUAAUAAUUGAGCCUUAAGAAGA
 AAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAUUCUAGUUUAUAGACAAGGC
 AAUCCUGAGCCAAGCCGAAGUAGUAAUUAAGUAAAGACCAGUGGACAAUCGACGGAUAACA
 GCAUAUCUAG

5'T302a-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-302a-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCCG
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
 CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
 CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCAGCAAG

UACAUCCACGUUUAAAGUGCCACCUAAAAACAGCCUGUGGGUUGAUCCACCCACAGGC
 CCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCC
 CUCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACC
 AGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAUAGACUGCUCA
 CGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAA
 CACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGA
 UGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCC
 AUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCU
 AGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACC
 UCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
 GGGUGUCCGUGUUUCAUUUUUAUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGA
 UCGUUAACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUUAUC
 CCUUUGUUGGGUUUAUACCACUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGU
 UAAGUUGAAUACAGCAAA**augggauccgugagcaagggcgaggagcuguuacccgggugggugccca**
uccgugcugagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauGCCA
ccuacggcaagcugaccugaaguucaucugcaccaccggcaagcugcccugcccuggcccaccucugugac
caccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuucucaagucc
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aggugaaguucgagggcgacaccucggugaaccgcaucgagcugaagggcaucgacuuaaggaggacggca
acaucuggggcacaagcuggaguacaacuaacagccacaacgucuauaaucauggccgacaagcagaagaa
cggcaucaaggugaacuuaagaucggccacaacaucgaggacggcagcgugcagcucgcccgaccacuaccag
cagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccuga
gcaaagacccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucgg
cauggacgagcuguacaagagaucucauaucaucgagugauagucuagaccuucugcggggcuugccuu
 cuggccaugcccuucucucuccuugcaccuguaccucuuuggucuuu**GAAUAAAGCCUGAGUAGGA**
AA
AA
AAAGGCUAUUAUGCGUUACCGGCGAGACGCU**ACGGACU****UAAAUAAUUGAGCCUAAAG**
AAGAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUUAUAGACA
AGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUGAUAAGACCAGUGGACAAUCGACGGAU
 AACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCC
 UGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3'T302a-5p Circular EGFP ΔpA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-302a-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**

GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUC AAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAAACGGUCGUGUGGGU**U**CAAGUCCUCCACCC**CC**ACGCCGGAACGCAAUAGC
 CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
 UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
 UAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCUCC**CC**CAACUGUAACUUAGAAG
 UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
 CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
 CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
 UUCGCUCAGCACUACCCAGUGUAGAUCAAGGUCGAUGAGUCACCGCAU**U**CCCCACGG
 GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
 CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
 GAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
 GUAACGGGCAACUCUGCAGCGGAACCGACUACU**U**UGGGUGUCCGUGUUCAUUUUUAU
 UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU**CG**UUACCAUAUAGCUAUUGGA
 UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
 UAGCUUGAAAGAGGUUAAAACAUAUACA**U**CAUUGUUAAGUUGAAUACAGCAAA**auggg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccugaaguucauc
ugcaccaccggcaagcugcccugcccugggccaccucugugaccaccucgaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggaagcagcggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacuucaagggaggacggcaaccuuccggggcacaagcugggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagaucgcca
caacaucgaggacggcagcgugcagcucgcccagccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagacccaacgagaagcgcgaucaucau
gguuccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucaucau
gcaucucgagugauag**AGCAAGUACAUCCAGUUUAAGU**ucuagaccuucugcggggcuugccuuc
uggccaugcccuucuucucccuugcaccuguaccuucuggucuuu**GAAUAAAGCCUGAGUAGGGG**
CUAUUAUGCGUUACCGGCGAGACGCU**ACGGACU**UAAAUAAUUGAGCCUAAAGAAGAA
 AUUCUUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAAU**CU**AGUUUAUAGACAAGGCA
 AUCCUGAGCCAAGCCGAAGUAGUAAU**U**AGUAA**GA**ACCAGUGGACAAUCGACGGAUAACAG
 CAUAUCUAG

3'T302a-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-302a-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAGAGAGGUAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUUCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAauggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cgggcacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugacccugaagucauc
ugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccucgaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcuguuucuaaguccgccaugcccgaaggcuacguccaggagcg
caccacuucuucaaggacgagcggaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacuuaagggaggacggcaacaucgggggcacaagcuggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuuaagaucgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaagaccccaacgagaagcgcgaucau
gguccugcuggaguucgugaccgcccgggaucacucuggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagAGCAAGUACAUCCACGUUUAAGUucuagaccuucugcggggcuugccuuc
uggccaugcccuucucucuccuugcaccuguaccucuuuggucuuuGAAUAAAGCCUGAGUAGGAA
AA
AA
AAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAUUAUUGAGCCUUAAGA
AGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAUUCUAGUUUAUAGACAA
GGCAAUCCUGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAGUGGACAAUCGACGGAUA
ACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCU

GCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

4x 5'T302a-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-302a-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGU**UGA**
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUC**AGCAAG**
UACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACGUU
UAAGUAGCAAGUACAUCCACGUUUAAGU**GCCACC****UUAAAACAGCCUGUGGGUUGAUC**
CCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCU
GUUUUAUACCCCCUCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCA
GCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUC
AAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCG
AAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUG
UAGAUCAAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGU
UGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAG
AGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGC
GGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGG
AACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUCCUAUACUGGCUGCUUAUGGU
GACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAG
AGCUAUUAUAUACCCUUUGUUGGGUUUAUACCACUAGCUUGAAAGAGGUUAAAACA
UUACAAUUCAUUGUUAAGUUGAAUACAGCAAA**augggauccgugagcaagggcgaggagcuguu**
caccggggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgga
ggggcgagggcgauGCCaccuacggcaagcugaccucgaagucaucugcaccaccggcaagcugcccugccc
uggcccaccucgugaccaccugaccuacggcgugcagugcuucagccguaccccgaccacaugaagcagc
acgacuucucucaaguccgccaugcccgaaggcuacguccaggagcgaccacuucucucaaggacgacggcaa
cuacaagacccgcgccgaggugaaguucgagggcgacaccucggugaaccgcaucgagcugaagggcaucgac
uucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucaugg
ccgacaagcagaagaacggcaucaaggugaacuuaagauccgccacaacaucgaggacggcagcgugcagcu
cgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagca
cccaguccgcccugagcaaaagacccaacgagaagcgcgaucaauugguccugcuggaguucgugaccgcccgc
cgggaucacucucggcauggacgagcuguacaagagaucucauauugcaucucgagugauagucuagaccuuc
ugcggggcuugccuucuggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAA
GCCUGAGUAGGAAA

AA
AAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCU**ACGGACU**AAAAUAAU
UGAGCCUUAAGAAGAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACC UAAUUC
UAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAAGUAAAGACCAGUGGA
CAAUCGACGGAUAAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCA
AGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

4x 3'T302a-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, **EGFP**, miR-302a-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUC**AAGACGAGGGU**AAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUAGGUCGAUGAGUCACCGCAUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUCCUAAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU**CGUUACCAU**AUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA**auggg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccugaaguucauc
ugcaccaccggcaagcugcccugcccuggcccaccucugugaccaccucgaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuaaucauggccgacaagcagaagaacggcaucaaggugaacuuaagaucgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgcacaaccacuaccugagcaccaguccgcccugagcaaaagacccaacgagaagcgcgaucau

gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
 gcaucucgagugauag**AGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAG**
UAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUucuagaccuucug
 cggggcuugccuucuggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGC
 CUGAGUAGGAA
 AA
 AAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCU**ACGGACU**UAAUAAUUG
 AGCCUAAAGAAGAAUUCUUUAAGUGGAUGCUCUCAAAACUCAGGGAAACCUAAUUCUA
 GUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAAGUAGAACAGGUGGACA
 AUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAG
 CUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

2x2 5'&3'T302a-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, **EGFP**, miR-302a-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUACAACUAAUCCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
 CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCACGCGGAAACGCAAUAGC
 CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUC**AGCAAG**
UACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUGCCACCUUAAAACAGCCU
 GUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACC
 UUUGUGCGCCUGUUUUUAUACCCCUCCCCCAACUGUAACUUAAGAAGUAACACACACCG
 AUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCG
 GACUGAGUAUCAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGC
 CAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCA
 CUACCCAGUGUAGAUCAGGUCGAUGAGUACCGCAUUCCCCACGGGCGACCGUGGC
 GGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGAC
 AUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUA
 AUCCUAAUCUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAA
 CUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAUCCUUAUACUGG
 CUGCUUAUGGUGACAAUUGAGAGAUUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCG
 GUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUUAAGCUUGAAAG
 AGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA**augggauccgugagcaag**
ggcgaggagcuguuacccgggguggugcccauccuggucgagcuggacggcgacgaaacggccacaaguuc
agcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccuugaagucaucugcaccaccggca
agcugcccuguccugggccaccucugugaccaccugaccuacggcgugcagugcuucagccgcuacccga

CCUUUGUUGGGUUUAUACCACUUGAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGU
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 uccuggucgagcugggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauGCCA
 ccuacgggaagcugacccugaaguucaucugcaccacgggaagcugcccugcccuggcccacccucgugac
 caccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuucucaagucc
 gccaugcccgaaggcuacguccaggagcgaccaucuucucaaggacgacggcaacuacaagacccgCGCCG
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 cggcaucaaggugaacucaagaucggccacaacaucgaggacggcagcgugcagcucgcccaccacuaccag
 cagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccuga
 gcaaagaccccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgcccggggaucaucucucgg
 cauggacgagcuguacaagagaucauauagcaucucgagugauagucuagaccuucugcggggcuugccuu
 cuggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGG
 GCUAUUAUGCGUUACCGGCGAGACGCU**ACGGACU**UAAAUAUUUGAGCCUAAAAGAAGA
 AAUUCUUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAAUCUAGUUUAGACAAGGC
 AAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACA
 GCAUAUCUAG

5'T21-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-21-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
 CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCCGGAACGCAAUAGC
 CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUC**UCAACA**
UCAGUCUGAUAGCUAGCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCACAGGC
 CCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCC
 CUCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACC
 AGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCA
 CGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAA
 CACCGUGGAAGUUGCAGAGUGUUUCGCUACGACUACCCAGUGUAGAUCAAGGUCGA
 UGAGUACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCC
 AUGGGGAAACCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCU
 AGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAAACUGCGGAGCACACACCC
 UCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
 GGGUGUCCGUGUUUCAUUUUUAUCCUUAUACUGGCUGCUUAUGGUGACAAUUGAGAGA

CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUACCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUUAUACCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA **auggg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauGCCUacggcaagcugaccugaagucauc
ugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuuca
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aaccgcaucgagcugaagggcaucgacuucaggaggagcggaacauccuggggcacaagcugggaguacaacu
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caacaucgaggacggcagcgugcagcucgcccagccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaagaccccaacgagaagcgcgaucau
gguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagUCAACAUCAGUCUGAUAAGCU **A** **ucua** **gaccuucugcggggcuugccuucu**
ggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGG
CUAUUAUGCGUUACCGGCGAGACGCU **ACGGACU** **U** **AAAAUAAUUGAGCCU** **U** **AAAGAAGAA**
AUUCUUUAAGUGGAUGCUCUCAAAACUCAGGGAAACCUAAAUCUAGUUUAUAGACAAGGCA
AUCCUGAGCCAAGCCGAAGUAGUAAUAGUAAG **ACCAGUGGACAAUCGACGGAUAACAG**
CAUAUCUAG

3'T21-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, **EGFP**, **miR-21-5p target site**

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC **AACAAUAGAUGACUUAACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAAGCUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGU **UGA**
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG

GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
 CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
 GAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
 GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
 UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
 UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
 UAGCUUGAAAGAGGUUAAAACAUAUACAAUUCAUUGUUAAGUUGAAUACAGCAAA **auggg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcugaccugaaguucauc
ugcaccaccggcaagcugcccugcccugggccaccucgugaccaccucgaccuacggcgugcagugcuuca
ggcgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggacgagggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacuucaaggaggagcggaacauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuaaucauaggccgacaagcagaagaacggcaucaaggugaacuuaagauccgccca
caacaucgaggacggcagcgugcagcucgcccagccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagacccaacgagaagcgcgaucauau
gguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauag **UCAACAUCAGUCUGAUAAGCUA** **aucuagaccuucugcggggcuugccuucu**
ggccaugcccuucucucuccuugcaccuguaccucuuuggucuuu **GAAUAAAGCCUGAGUAGGAAA**
AA
AA
AGGCUAUUAUGCGUUAACCGGCGAGACGCU **ACGGACUUA** **AAUUAUUGAGCCUUA** **AAAGAA**
GAAUUCUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAG
GCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAAGUAG **ACCAGUGGACAAUCGACGGAUAA**
CAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUG
CAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

5'T339-5p Circular EGFP ΔpA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-339-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC **AACAAUAGAUGACUUAACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUC **AAGACGAGGGUAAAGAGAGAG**
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG **AAAAUCCGU** **UGA**
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUC **cgugagcu**
ccuggaggacagggg **GCCACC** **UUA** **AAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUU**
GGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCC

CCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCA
CGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCG
GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACC
GUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAG
UCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGG
GGAAACCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUU
GGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAAACUGCGGAGCACACACCCUCAA
GCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGU
GUCCGUGUUUCAUUUUUAUCCUUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGU
UACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUU
UGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAG
UUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguuacccgggguggugcccauccug
gucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuac
ggcaagcugaccguagaugucaucugcaccaccggcaagcugcccuguccugggcccaccucugugaccaccc
ugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuucuucaaguccgccau
gcccgaaggcuacguccaggagcgacccaucuucaagggacgacggcaacuacaagacccgcgcccaggug
aaguucgagggcgacaccuggugaaccgcaucgagcugaagggcaucgacuuaaggaggacggcaacauc
uggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcau
caaggugaacuuaagauccgccacaacaucgaggacggcagcgugcagcucgcccaccacuaccagcagaac
accccaucggcgacggccccgugcugcugcccgcacaaccacuaccugagcaccaguccgcccugagcaaag
accccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucggcaugga
cgagcuguacaagagaucauauugcaucucgagugauagucuaagaccuucugcggggcuugccuucuggcc
augcccuucucucuccuugcaccuguaccucuuggucuuu**GAAUAAAGCCUGAGUAGGGGCUAU**
UAUGCGUUACCGGCGAGACGCU**ACGGACU**UAAAUAAUUGAGCCUAAAAGAAGAAAUUC
UUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAAUUCUAGUUAUAGACAAGGCAAUCC
UGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUA
UCUAG

5'T339-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-339-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCC**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGU**UGA**
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUC**cgugagcu**
ccuggaggacagggg**GCCACC****U**AAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAU

GGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCC
CCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCA
CGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCG
GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACC
GUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAG
UCACCGCAUUCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGG
GGAAACCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUU
GGUAGUCCUCCGGCCCCUGAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAA
GCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGU
GUCCGUGUUUCAUUUUUAUUCUUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGU
UACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUU
UGUUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAACAUUACAAUUAUUGUUAAG
UUGAAUACAGCAAA**augggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccug**
gucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuac
ggcaagcugaccgugaagucaucugcaccaccggcaagcugcccuguccggccaccucugugaccaccc
ugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuucuucaaguccgccau
gcccgaaggcuacguccaggagcgaccacuucuucaaggacgacggcaacuacaagaccgcgcccaggug
aaguucgagggcgacaccuggugaaccgcaucgagcugaagggcaucgacuucaggaggacggcaacauc
uggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcau
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accccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaag
accccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucggcaugga
cgagcuguacaagagaucauauugcaucucgagugauagucuaagaccuucugcggggcuugccuucuggcc
augcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAA
AA
AAAGG
CUAUUAUGCGUUACCGGCGAGACGCU**ACGGACU**AAAUAAUUGAGCCUUAAGAAGAA
AUUCUUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAAUCUAGUUUAUAGACAAGGCA
AUCCUGAGCCAAGCCGAAGUAGUAAUUAAGUAGAACAGUGGACAAUCGACGGAUAACAG
CAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAG
GUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3'T339-5p Circular EGFP ΔpA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-339-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG

UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
 CCUUAACGGUCGUGUGGGUUAAGUCCCUCACCCCCACGCCGGAACGCAAUAGC
 CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
 UUAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
 UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCCAACUGUAACUUAGAAG
 UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
 CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
 CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
 UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
 GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
 CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
 GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
 GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAU
 UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUUCGUUACCAUAUAGCUAUUGGA
 UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
 UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA **auggg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cgggcacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugacccugaagucauc
ugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccucgaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcucuucuaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacuucuaagggaggacggcaacauccuggggcacaagcugggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuuaagauccgcca
caacaucgaggacggcagcgugcagcugccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagaccccaacgagaagcgcgaucaacu
gguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagcgugagcuccuggaggacagggaucuaagaccuucugcggggcuugccuucuggcca
ugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAU
UAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAUUGAGCCUAAAAGAAGAAAUUC
 UUUAAUGUGGAUGCUCUCAACUCAGGGAAACCUAAAUCUAGUUUAUAGACAAGGCAAUCC
 UGAGCCAAGCCGAAGUAGUAAUUAAGUAAAGACCAGUGGACAAUCGACGGAUACAGCAUA
 UCUAG

3'T339-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-339-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCACAAUAGAUGACUUACAACUAAUCCG

GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUCAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUA AACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUAGGUCGAUGAGUCACCGCAUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU CGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cgggcacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcugaccugaaguucauc
ugcaccaccggcaagcugcccugcccugggccaccucugugaccaccucgaccuacggcgugcagugcuuca
gcccguaccccgaccacaugaagcagcagcagcuucucaaguuccgccaugcccgaaggcuacguccaggagcg
caccuacuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaauccuggggcacaagcugggaguacaacu
acaacagccacaacgucuaaucauaggccgacaagcagaagaacggcaucaaggugaacuuaagaucgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgccugagcaaaagacccaacgagaagcgcgaucauau
gguccugcuggaguucgugaccgccgcccgggaucacucucggcauggacgagcuguacaagagaucauau
gcaucucgagugauagcgugagcuccuggaggacagggaucuagaccuucugcggggcuugccuucuggcca
ugcccuucucucuccuugcaccuguaccucuuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAA
AA
AAAGGC
UAUUAUGCGUUACCGGCGAGACGCUACGGACUUAUUAAUUGAGCCUUAAGAAGAAA
UUCUUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAAUCUAGUUUAAGACAAGGCAA
UCCUGAGCCAAGCCGAAGUAGUAAUUAUAGUAGACCAGUGGACAAUCGACGGAUAACAGC
AUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGG
UCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

5'T17-5p Circular EGFP ΔpA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-17-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGU**UGA**
CCUAAAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUC**cuaccugc**
acuguaagcacuuugGCCACC**UAAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAU**
GGGCGCUAGCACUCUGGUUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCC
CCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCA
CGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCG
GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACC
GUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAG
UCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGG
GGAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUU
GGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAAACUGCGGAGCACACACCCUCAA
GCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGU
GUCCGUGUUUCAUUUUUAUCCUUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU
CGUUAACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCU
UGUUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAG
UUGAAUACAGCAAA**auggggauccgugagcaagggcgaggagcuguuacccgggguggugcccauccug**
gucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuac
ggcaagcugaccugaaguucaucugcaccaccggcaagcugcccugcccuggcccaccuccgugaccaccc
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accccaacgagaagcgcgaucauagguccugcuggaguucgugaccgcccgggaucacucucggcaugga
cgagcuguacaagagaucauauagcaucucgagugauag**ucuagaccuucugcggggcuugccuucuggcc**
augcccuucucucuccuugcaccuguaccucuuggucuuu**GAAUAAAGCCUGAGUAGGGGCUAU**
UAUGCGUUACCGGCGAGACGCU**ACGGACU****UAAAUAUUUGAGCCUAAAAGAAGAAAUUC**
UUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCC
UGAGCCAAGCCGAAGUAGUAAUAGUAAG**ACCAGUGGACAAUCGACGGAUAACAGCAUA**

UCUAG

5'T17-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-17-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUC**cuaccugc**
acuguaagcacuuugGCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAU
GGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCUCCC
CCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCA
CGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCG
GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACC
GUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAG
UCACCGCAUUCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGG
GGAAACCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUU
GGUAGUCCUCCGGCCCCUGAAUGCGGCUAUCCUAAACUGCGGAGCACACACCCUCAA
GCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGU
GUCCGUGUUUCAUUUUUAUCCUUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU
UACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCU
UGUUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAG
UUGAAUACAGCAAA**augggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccug**
gucgagcuggacggcgacguaaacggccacagaagucagcguguccggcgaggggcgaggggcgaugccaccuac
ggcaagcugaccgugaaguucaucugcaccaccggcaagcugcccuguccugggccaccucgugaccacc
ugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuucuucaaguccgcca
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caaggugaacuucagaugccgccacaacaucgaggacggcagcugcagcucgcccaccacuaccagcagaac
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acccaacgagaagcggaucacaugguccugcuggaguucgugaccgcccgggaucacucucggcaugga
cgagcuguacaagagaucauauugcaucucgagugauagucuagaccuucugcggggcuugccuucuggcc
augcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAA
AA
AAAGG

CUAUUAUGCGUUACCGGCGAGACGCU**ACGGACU**UAAAUAUUUGAGCCUAAAAGAAGAA
AUUCUUUAAGUGGAUGCUCUCAACUCAGGGAAACCUAAAUCUAGUUUAAGACAAGGCA
AUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAG
CAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAG
GUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3'T17-5p Circular EGFP ΔpA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-17-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCU**AACGUCAAGACGAGGGU**AAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUA**AAACGGUCGUGUGGGU**UCAAGUCCCUCCACCC**CCACGCCG**GAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCC**CCCAACUGUAACU**UAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAAGGUCGAUGAGUCACCGCAU**UCCCCACGG**
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAU**GGGGAACCC**AUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUA**AUCCUAACUGCGGAGCACACACCCU**CAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACU**UUGGGUGUCCGUGU**UCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU**CGUUACCAU**AUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUUAU**UCCCUU**GUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAU**UCAUUGU**UAAGUUGAAUACAGCAA**auagg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcugaccugaagucauc
ugcaccaccggcaagcugcccugcccugggccaccucugagaccaccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
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acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuuaagauccgcca
caauaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
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gguccugcuggaguucgugagccgcgcccgggaucacucucggcauggacgagcuguacaagagaucucauau

gcaucucgagugauagcuaccugcacuguaagcacuuugucuagaccuucugcggggcuugccuucuggcca
ugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAU
UAUGCGUUACCGGCGAGACGCU**ACGGACU**UAAUAAUUGAGCCUAAAAGAAGAAAUUC
UUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUUAGUUUAUAGACAAGGCAAUCC
UGAGCCAAGCCGAAGUAGUAAUUAAGUAAAGACCAGUGGACAAUCGACGGAUAACAGCAUA
UCUAG

3'T17-5p Circular EGFP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, miR-17-5p target site

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUCAGAGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUAACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA**augggg**
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccugaagucauc
ugcaccaccggcaagcugcccugcccuggcccaccucugugaccaccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuaaucauggccgacaagcagaagaacggcaucaaggugaacuuaagaucgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagacccaacgagaagcgcgaucau

3'T206 Linear EGFP

GGG**AAAAUCCG**U GACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAGUAAGAAGAAAUUAAGACAC
CGGUCGCCACC**UAAAA**CAGCCUGUGGGUUGAUCC**ACCCAC**CAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGU
AACUUGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUAACCCGGACUGAGUAUCAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUU
AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAA**augggaucc**
gugagcaagggcgagggagcuguuacccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaag
uucagcguguccggcgagggcgagggcgauccaccuacggcaagcugacccugaagucaucugcaccaccgggaagc
ugcccgugcccgugccaccucgugaccaccugaccuacggcgugcagugcuucagccgcuaacccgaccacaugaa
gcagcacgacuucucaaguccgcaugcccgaaggcuacguccaggagcgaccaucuucucaaggacgacggcaac
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gaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaaucauggccgacaagcag
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agaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaaga
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ggcuugccuucuggccaugcccuucucucuccuugcaccguaccucuuggucuuuGAAUAAAGCCUGAGUA
GGGGCUAUUAUGCGUUAACGGCGAGACGCU**ACGGACUU**AAAAAAAAAAAAAAAAAAAAAAAA

AA
AA

5'T302a-5p Linear EGFP

Group I intron, CVB3 IRES, EGFP, miR-302a-5p target site

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACAC
CGGUCAGCAAGUACAUCACGUUUAAGUGCCACCUUAAAACAGCCUGUGGGUUGAUCCC
ACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUU
UUUAUACCCCCUCCCCAACUGUAACUAGAAGUAACACACACCGAUCAACAGUCAGCGU
GGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGA
CUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCU
AGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGG
UCGAUGAGUCACCGCAUUCGCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCU
GCCAUGGGGAAACCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGA
GCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAUCCUAACUGCGGAGCACACAC
CCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUU
UGGGUGUCCGUGUUUCAUUUUUAUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGA
UCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUACC
CUUUGUUGGGUUUAUACCACUUGAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUA
AGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguuacccgggguggugcccauccugguc
gagcuggagcggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcug
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cggcagcgugcagcucgcccaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacu
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ggggcuugccuucuggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAG
UAGGGGCUAUUAUGCGUUAACGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAA
AA
AA

3'T302a-5p Linear EGFP

Group I intron, CVB3 IRES, EGFP, miR-302a-5p target site

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGU
AACUUAGAAGUAACACACACCCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUU
AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggaucc
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ugcccgucccugggcccaccucgugaccaccugaccuacggcgugcagugcuucagccguaccccgaccacaugaa
gcagcacgacuucucaaguccgccaugcccgaaggcuacguccaggagcgacccaucuucucaaggacgacggcaac
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gaggacggcaacaucuggggcacaagcuggaguacaacuacaacagccacaacgucuauaauaugggcgacaagcag
aagaacggcaucaaggugaacuuaagaauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagc
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cccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcug
uacaagagaucauauaugcaucucgagugauagAGCAAGUACAUCACGUUUAAGUucuagaccuucugcg
gggcuugccuucugggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGU
AGGGGCUAUUAUGCGUUAACGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAA
AA
AA

5'T21-5p Linear EGFP

Group I intron, CVB3 IRES, EGFP, miR-21-5p target site

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACAC
CGGUCUACAACUAGUCUGAUAAAGCUAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCA

CCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUU
 UAUACCCCUCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUG
 GCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGAC
 UGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUA
 GUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGU
 CGAUGAGUCACCGCAUUCACCGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUG
 CCCAUGGGGAAACCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAAUUGAG
 CUAGUUGGUAGUCCUCCGGCCCCUGAAUUCGGCUAAUCCUAACUGCGGAGCACACACC
 CUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
 GGGUGUCCGUGUUUCAUUUUAUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU
 CGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCC
 UUUGUUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAACAUAACAAUUAUUGUAA
 GUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcguuacacgggguggugcccauccgguccg
 agcuggagcggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcuga
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 cgcaccaucuuucaaaggcagcagggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggugaacc
 gcaucgagcugaagggcaucgacuuaaggaggagcggcaacaucuggggcacaagcuggaguacaacuacaacagcc
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 gcagcgugcagcucgcccagccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuac
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 ccgggaucacucucggcauggagcagcuguacaagagaucucuaugcaucucgagugauagucuagaccuucugcgg
 ggcugccuucuggccaugcccuucucucuccuugcaccguaccucugugucuuGAAUAAAGCCUGAGUA
 GGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAA
 AA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

3'T21-5p Linear EGFP

Group I intron, CVB3 IRES, EGFP, miR-21-5p target site

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCC
 GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACAC
 CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCU
 AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGU
 AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
 CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
 AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA

GAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUU
AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA **augggaucc**
gugagcaagggcgaggagcuguuacacgggguggugcccuccuggucgagcuggacggcgacguaaacggccacaag
uucagcguuguccggcgaggcgaggcggaugccaccuacggcaagcugaccuagaagucaucugcaccaccgggaagc
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gaggacggcaacaucuggggcacaagcuggaguacaacuacaacagccacaacgucuauaaucauggccgacaagcag
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ccccaacgagaagcgcgaucaauguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcug
uacaagagaucauauugcaucucgagugauag **UCAACAUCAGUCUGAUAAGCUA** **ucuagaccuucugcg**
ggcuugccuucuggccaugcccuucucucuccuugcaccuguaccucuuggucuuu GAAUAAAGCCUGAGUA
GGGGCUAUUAUGCGUUACCGGCGAGACGCU **ACGGACUU** AAAAAAAAAAAAAAAAAAAAAA
AA
AAA

5'T339-5p Linear EGFP

Group I intron, CVB3 IRES, EGFP, miR-339-5p target site

GGG**AAAAUCCGU**UGACCUUAAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACAC
CGGUC**cgugagcuccuggaggacaggga**GCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCA
CAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUA
CCCCUCCCCAACUGUAACUUAAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCAC
ACCAGCCACGUUUUGAUCAAGCACUUCUGUUAACCCGGACUGAGUAUCAAUAGACUGCU
CACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAA
CACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAU
GAGUACCGCAUUCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCA
UGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAG
UUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCA

AGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGU
 GUCCGUGUUUCAUUUUUAUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUU
 ACCAUUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUG
 UUGGGUUUAUACCACUUAAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUG
 AAUACAGCAAAaugggauccgugagcaagggcgaggagcguuacacgggguggugcccuccgugcagcugg
 acggcgacguaaacggccacaaguucagcguugccggcgagggcgagggcgauGCCaccuacggcaagcugaccuaga
 aguucacugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuu
 cagccgcuaccccgaccacaugaagcagcagcguuucuaaguccgccaugcccgaaggcuacguccaggagcgcacc
 aucuucuaaggaagcagcggcaacuacaagaccgcgcccaggguagaaguucgagggcgacaccucggugaaccgcauc
 gagcugaagggcaucgacuuaaggaggacggcaacuaccuggggcacaagcuggaguacaacuacaacagccacaac
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 GGCUAUUAUGCGUUAACGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AA
 AA

3'T339-5p Linear EGFP

Group I intron, CVB3 IRES, EGFP, miR-339-5p target site

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCC
 GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACAC
 CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCU
 AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGU
 AACUUAGAAGUAACACACACCCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
 CAAGCACUUCUGUUACCCCGGACUGAGUAUCAUAGACUGCUCACGCGGUUGAAGGAG
 AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
 GAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUC
 CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUUGG
 ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
 CCCUGAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
 GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUU
 AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUUAUAGCUAUUGGA
 UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
 AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggaucc

gugagcaagggcgaggagcuguuacccgggguggugcccauccuggugcagcuggacggcgacguaaacggccacaag
uucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccuugaaguucaucugcaccaccgggaagc
ugcccuguccugggccaccucgugaccaccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaa
gcagcacgacuucucaaguccgccauGCCgaaggcuacguccaggagcgcaccaucuucucaaggacgacggcaac
uacaagaccgcgcccagguagaaguucgagggcgacaccugguagaaccgcaucgagcugaagggaucgacuuaag
gaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcag
aagaacggcaucaaggugaacuuaagaucggccacaacaucgaggacggcagcgugcagcucgcccaccacuaccagc
agaacacccccaucggcgacggccccgugcugcugcccgaaccacuaccugagcaccaguccgcccugagcaaaaga
ccccaacgagaagcggaucacaugguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcug
uacaagagaucucauauagcaucucgagugauagcgugagcuccggaggacagggaucuagaccuucugcggggcuug
ccuucuggccaugcccuucucucccuugcaccguaccucuuuggucuuuGAAUAAAGCCUGAGUAGGGG
CUAUUAUGCGUUAACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AA

linear switch (miR-206)

EGFP, miR-206 target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcCCACACACU
UCCUUAUUAUCCAgccaccaugggauccgugagcaagggcgaggagcuguuacccgggguggugcccauccugg
ucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagc
ugaccuugaaguucaucugcaccaccggcaagcugcccugcccuggccaccucgugaccaccugaccuacggcgug
gcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccauGCCgaaggcuacguccag
gagcgcaccaucuucucaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggaucgacuuaaggagggacggcaacaucuggggcacaagcuggaguacaacuacaac
agccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuuaagaucggccacaacaucgag
gacggcagcgugcagcucgcccaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgaacc
acuaccugagcaccaguccgcccugagcaaaagaccccaacgagaagcgcgaucaauguccugcuggaguucgugac
cgccgcccgggaucacucucggcauggacgagcuguacaagagaucauauagcaucucgagugauagucuagaccuuc
ugcggggcuugccuucuggccaugcccuucucucccuugcaccguaccucuuuggucuuuGAAUAAAGCCUG
AGUAGGAA
AA

linear switch (miR-302a-5p)

EGFP, miR-302a-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcAGCAAGUAC
AUCCACGUUUAGUgccaccaugggauccgugagcaagggcgaggagcuguuacccgggguggugcccauccug

gucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaag
cugaccugaaguucaucugcaccaccggcaagcugcccugcccuggcccaccucugugaccaccugaccuacggcg
ugcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugccgaaggcuacgucca
ggagcgcaccaucuucucaaggacgacggcaacuacaagaccgcgcccagggugaaguucgagggcgacaccuggu
gaaccgcaucgagcugaagggaucgacuuaaggaggacggcaacuuccuggggcacaagcuggaguacaacuacaa
cagccacaacgucuauaaucauggccgacaagcagaagaacggcaucaaggugaacuuaagauccgccacaacauca
ggacggcagcgugcagcucgcccaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaac
cacuaccugagcaccaguccgcccugagcaaagaccccaacgagaagcgcgaucauagguccugcuggaguucguga
ccgcccggggaucaucucggauggacgagcuguacaagagaucauauagcaucucgagugauagucuagaccuu
cugcggggcuugccuucuggccaugcccuucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCU
GAGUAGGAAA
AAA
A

linear switch (miR-21-5p)

EGFP, miR-21-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcUCAACAUC
GUCUGAUAAAGCUAgccaccaugggauccgugagcaagggcgaggagcuguuacccgggugugcccuccug
ucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagc
ugaccugaaguucaucugcaccaccggcaagcugcccugcccuggcccaccucugugaccaccugaccuacggcg
gcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuucucaaguccgccaugccgaaggcuacguccag
gagcgcaccaucuucucaaggacgacggcaacuacaagaccgcgcccagggugaaguucgagggcgacaccucggug
aaccgcaucgagcugaagggaucgacuuaaggaggacggcaacuuccuggggcacaagcuggaguacaacuacaac
agccacaacgucuauaaucauggccgacaagcagaagaacggcaucaaggugaacuuaagauccgccacaacauca
gacggcagcgugcagcucgcccaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaacc
acuaccugagcaccaguccgcccugagcaaagaccccaacgagaagcgcgaucauagguccugcuggaguucgugac
cgcccggggaucaucucggauggacgagcuguacaagagaucauauagcaucucgagugauagucuagaccuuc
ugcggggcuugccuucuggccaugcccuucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUG
AGUAGGAAA
AAA

linear switch (miR-339-5p)

EGFP, miR-339-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcggugagcuccug
gaggacagggagccaccaugggauccgugagcaagggcgaggagcuguuacccgggugugcccuccuggucgagc
uggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggcaagcugaccc

ugaaguucaucugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccucgaccuacggcgugcagug
cuucagccgcuaccccgaccacaugaagcagcacgacuuuuaaguccgccaugcccgaaggcuacguccaggagcgc
accaucuucuuaaggacgacggcaacuacaagaccgcgcgaggugaaguucgagggcgacaccugguagaaccgc
aucgagcugaagggcaucgacuuaaggaggacggcaacuuccuggggcacaagcuggaguacaacuacaacagccac
aacgucuauaaucauggccgacaagcagaagaacggcaucaaggugaacuuaagauccgccacaacaucgaggacggc
agcgugcagcucgcccaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccu
gagcaccaguccgcccugagcaaagaccccaacgagaagcgcgaucaauguccugcuggaguucgugaccgcccgc
gggaucacucucggcauggacgagcuguacaagagaucucauauaagcaucucgagugauagucuagaccuucugcgggg
cuugccuucuggccaugcccuucucucucccuugcaccguaccucuuggucuuuGAAUAAAGCCUGAGUAG
GAAA
AAA

MS2CP responsive Circular EGFP variant1 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, MS2CP binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCGGGAGCAGGUGAGGAUCACCCAUCUGCCACGAGCGAGGUGAGGAUCAC
CCAUCUCGUCUGUUC**CUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGU**
GGAAGUUGCAGAGUGUUUCGUCAGCACUACCCAGUGUAGAUCAAGGUCGAUGAGUC
ACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGG
AAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGG
UAGUCCUCCGGCCCCUGAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGC
CAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGU
CCGUGUUUCAUUUUAUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUUGUUA
CCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUG
UUGGGUUUAUACCACUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUU
GAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccugguc
gagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuacggc
aagcugaccugaaguucaucugcaccaccggcaagcugcccugcccuggcccaccucgugaccaccuga
ccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuuaaguccgccaugcc

cgaaggcuacguccaggagcgcaccaucuucucaaggacgacggcaacuacaagacccgcgccgaggugaag
 uucgagggcgacaccucggugaaccgcaucgagcugaagggcaucgacucaaggaggacggcaacaucugg
 ggcacaagcuggaguaacaacaacagccacaacgucuaaucauggccgacaagcagaagaacggcaucaa
 ggugaacuuaagaucgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacc
 cccaucggcgacggccccgugcugcugccccgacaaccacuaccugagcaccaguccgccugagcaaagacc
 ccaacgagaagcgcgaucaucaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacga
 gcuguacaagagaucauauugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaug
 cccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUA
UGC**G**U**A**CC**G**GC**G**AG**A**CG**C**U**A**CG**G**AC**U**U**A**AA**U**AA**U**UG**A**GC**C**U**A**AA**G**A**A**GA**A**AA**U**CU**U**
 U**A**AG**U**GG**A**UG**C**UC**U**CAA**A**C**U**CAG**G**GA**A**CC**U**AA**A**UC**U**AG**U**U**A**U**A**G**A**CA**A**GG**C**AA**U**CC**U**G
 A**G**CC**A**AG**C**CG**A**AG**U**AG**U**AA**U**AG**U**A**A**GACCAGUGGACAAUCGACGGAUAAACAGCAUAUC
 UAG

MS2CP responsive Circular EGFP variant2 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, MS2CP binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACU**UACAACUAAUCG
 GAAGGUGCAGAGACUC**GACGGAGCU**ACCCU**AACGU**CAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGU**UGA
CCUUAAACGGUCGUGUGGGU**U**CAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
 CGGCGAAU**U**AAGAGAGAAAAGAAGAGUAAGAAGAAA**U**AUAAGACACCGGUCGCCACC
 U**U**AA**A**ACAGCCUGUGGGU**GGUGAGGAUCACCCAUC**CACCCACAGGCCCGGTGAGGAT
CACCCATCGGGCGCTAGCACTCTGGTAG**GGUGAGGAUCACCCAUC**UACCUUUGUGCGCC
 UGUUUU**A**UACCCCUCCCCAACUGUAACU**U**AGAAGUAACACACACCGAUCAACAGUC
 AGCGUGGCACACCAGCCACGUUU**U**GAUCAAGCACUUCUGU**U**ACCCCGGACUGAGU**U**
 CAAUAGACUGCUCACGCGGU**U**GAAGGAGAAAGCGUUCGU**U**AUCCGGCCAACUACUUC
 GAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU**U**UCGCUCAGCACUACCCAGU
 GUAGAU**C**AGGUCGAUGAGUCACCGCAU**U**CCCCACGGGCGACCGUGGCGGUGGCUGCG
 UUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCU**U**AAUACAGACAUGGUGCGAA
 GAGUCU**U**AUUGAGCUAGUUGGUAGUCCUGGCCCCUGAAUGCGGC**U**AAUCCU**U**AAUCUG
 CGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGU**U**AACGGGCAACUUGCAGCG
 GAACCGACUACU**U**UGGGUGUCCGUGU**U**UCAU**U**U**U**AUCCU**U**AUACUGGCUGCU**U**AUGG
 UGACAAUUGAGAGAU**C**GUUACCAU**U**AGCU**U**AUUGGAUUGGCCAUCCGGUGACU**U**AAU**A**
 GAGCU**U**U**U**AU**U**AUCCCU**U**UGUUGGGU**U**U**U**AUACCACU**U**AGCU**U**GAAAGAGGU**U**AA**A**AC
 AU**U**ACAAU**U**CAUUGU**U**AAGU**U**GAAUACAGCAAA**augggauccgugagcaagggcgaggagcugu**
ucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcg
agggcgagggcgauGCCaccuacggcaagcugaccguagaugucaucugcaccaccggcaagcugccccgugcc

cuggcccaccucgugaccaccucgaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcag
cacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuuuucaaggacgacggca
acuacaagaccgcgcccaggugaaguucgagggcgacaccucggugaaccgcaucgagcugaaggggaucga
cuucaaggaggacggcaacaucugggggcacaagcuggaguacaacuacaacagccacaacgucuaaucaug
gccgacaagcagaagaacggcaucaaggugaacuucagaucgccacaacaucgaggacggcagcugcagc
ucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagc
accaguccgcccugagcaagaccccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgccg
ccgggaucacucucggcauggacgagcuguacaagagaucucauauugcaucucgagugauagucuagaccuu
cugcgggggcuugccuucuggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAA
AGCCUGAGUAGGGGCUAUUAUGCGUUAACGGCGAGACGCUACGGACUUAAAUAUUUGA
GCCUAAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAAACUCAGGGAAACCUAAAUCUAG
UUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAA
UCGACGGAUAACAGCAUAUCUAG

MS2CP responsive Circular EGFP variant3 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, MS2CP binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCGGCCCCU
GAAUGCGGCUAUUCUAACUGCGGAGCACACACCCUCGCAGGUGAGGAUACCCAUC
UGCCACGAGCGAGGUGAGGAUACCCAUCUCGUCUGUGAGGGCAGUGUGUCGUAA
CGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAUUCU
AUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGG
CCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUUAGC
UUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccg
ugagcaagggcgaggagcuguuacccgggguggugcccauccuggucgagcuggacggcgacguaaacggcc

acaaguucagcgcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccucgaagucaucugcac
caccgggaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuucagccgc
uaccccgaccacaugaagcagcacgacuucuuaaguccgccaugcccgaaggcuacguccaggagcgcacca
ucuucuuaaggacgacggcaacuacaagacccgcgagggagguagaaguucgagggcgacaccucggugaaccg
caucgagcugaagggcaucgacuuaaggaggacggcaacaucggggcacaagcuggaguacaacuacaac
agccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuuaagauccgccacaaca
ucgaggacggcagcgcgucgagcucgcccaccacuaccagcagaacacccccaucggcgagggccccgugcugcu
gcccgaacaaccacuaccugagcaccaguccgcccugagcaaagaccccaacgagaagcgcgaucacauggucc
ugcuggaguuucgugaccgcccgcgggaucacucucggcauggacgagcuguacaagagauucuaugcauc
ucgagugauagucuaagaccuucugcggggcuugccuucuggccaugcccuucucuccuugcaccugua
ccucuuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUUAUGCGUUACCGGCGAGACGCUA
CGGACUUAAAUAUUUGAGCCUUAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAACUCA
GGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUA
GUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

MS2CP responsive Circular EGFP variant4 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, MS2CP binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGCAGGUGAGGAUACCCAUCUGCCAC
GAGCGAGGUGAGGAUACCCAUCUCGUCUGUGUUGAUCGUUACCAUAUAGCUAUUG
GAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACC
ACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAa

ugggauccgugagcaagggcgaggagcuguucacccgggguggugcccauccuggucgagcuggacggcgacg
 uaaacggccacaaguucagcguguccggcgagggcgagggcgauccaccuacgggaagcugaccugaauguu
 caucugcaccaccgggaagcugcccugcccuggcccaccucugugaccaccucgaccuacggcgugcagugc
 uucagccgcuaccccgaccacaugaagcagcagcagacuucucaaguccgccaugcccgaaggcuacguccagg
 agcgcaccaucucucaagggacgagcggaacuacaagacccgcgcccaggugaaguucgagggcgacacccu
 ggugaaccgcaucgagcugaagggcaucgacuucagggaggacggcaauccuggggcacaagcuggaguac
 aacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucagauc
 gccacaaucaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgagggcccc
 gugcugcugcccgacaaccacuaccugagcaccaguccgccugagcagaagaccccaacgagaagcgcgau
 caaugguccugcuggaguucgugaccgccgcccgggaucacucucggcauggacgagcuguacaagagaucuc
 auaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucuccuu
 gcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCG
AGACGCUACGGACUUAAAUAUUGAGCCUUAAGAAGAAAUUCUUAAGUGGAUGCUCU
 CAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGU
 AGUAAUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

U1A responsive Circular EGFP variant1 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, U1A binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUAACAACUAAUCG
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCGACAGCAUUGUACCCAGAGUCUGUCCCGAGACAUUGCACCUGGCGCUG
UCUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCGCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUACCUAACUGCGGAGCACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUUGUUAACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAuggg

auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
 cggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugacccugaaguucau
 ugcaccaccgggaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuuca
 gccgcuaccccgaccacaugaagcagcacgacuucuuaaguccgccaugcccgaaggcuacguccaggagcg
 caccacuucuuaaggacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccucggug
 aaccgcaucgagcugaagggcaucgacuuaaggaggacggcaacaucuggggcacaagcugggaguacaacu
 acaacagccacaacgucuauaaucauggccgacaagcagaagaacggcaucaaggugaacuuaagauccgcca
 caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
 cugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagaccccaacgagaagcgcgaucau
 gguccugcugggaguucgugaccgcgcccgggaucacucucggcauggacgagcuguacaagagaucucauau
 gcaucucgagugauagucuagaccuucugcgggggcuugccuucuggccaugcccuucucucuccuugcac
 cuguaccucucuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGAC
GCUACGGACUUAAAUAUUGAGCCUUAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAA
 CUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUA
 AUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

U1A responsive Circular EGFP variant2 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, U1A binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCC
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAUUGUACUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCAUGGGACGCU
CUAAUACAGACAUGGUGAUUGCACGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCC
UGAAUGCGGCUAAUCCUAACUGCGGAGCACACCCUCAAGCCAGAGGGCAGUGUGU
CGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGUGUCCGUGUUUCAUUUUA
UUCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGG
AUUGGCAUCCGGUGACUAAUAGAGCUAUUAUAUACCCUUUGUUGGGUUUAUACCAC
UUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaug

ggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacgua
 aacggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccugaaguua
 ucugcaccaccgggaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuu
 cagccgcuaccccgaccacaugaagcagcagcagcguuucuaaguccgccaugcccgaaggcuacguccaggag
 cgcaccaucuuuuaagggagcagggcaacuacaagaccggcgccgaggugaaguucgagggcgacaccucgg
 ugaaccgcaucgagcugaagggcaucgacuucaaggaggagcggcaacaucuggggcacaagcugggaguacaa
 cuacaacagccacaacgucuaaucauaggccgacaagcagaagaacggcaucaaggugaacuuaagaucggc
 cacaacaucgaggagcggcagcgugcagcucgcccaccacuaccagcagaacacccccaucggcgacggccccg
 ugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagaccccaacgagaagcgcgauac
 augguccugcugggaguucgugaccggcgccgggaucacucucggcauggagcagcuguacaagagaucucau
 augcaucucgagugauagucuagaccuucugcgggggcuugccuucuggccaugcccuucucucucccuugc
 accuguaccucugugucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAG
ACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCA
 AACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAG
 UAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

U1A responsive Circular EGFP variant3 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, U1A binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCCG
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUUCUAACUGCGGAGCACACACCCUCACAGCAUUGUACCCAGAGUC
UGUCCCCAGACAUUGCACCUGGCGCUGUGAGGGCAGUGUGUCGUAACGGGCAACUCU
GCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAUUCUAUACUGGCUGC
UUAUGGUGACAAUUGAGAGAUUGUUAACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGA
CUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGU

UAAACAUAUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgag
 gaggcuguuacacggggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcgug
 uccggcgagggcgagggcgauGCCaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugc
 ccgugcccugggccaccucgugaccaccugaccuacggcgugcagugcuucagccguaccccgaccacau
 gaagcagcacgacuucucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucucaaggac
 gacggcaacuacaagacccgcgccgaggugaaguucgagggcgacaccucggugaaccgcaucgagcugaagg
 gcaucgacuucaggaggacggcaacaucggggcacaagcuggaguacaacuacaacagccacaacgucua
 uaucauggccgacaagcagaagaacggcaucaaggugaacuucagaucggccacaacaucgaggacggcagc
 gugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacu
 accugagcaccaguccgcccugagcaaagaccccaacgagaagcgcgaucauagguccugcuggaguucgu
 gaccgccgccgggaucacucucggcauggacgagcuguacaagagaucauauugcaucucgagugauaguc
 uagaccuucugcggggcuugccuucuggccaugcccuucucucccuugcaccuguaccucuugggucuuu
GAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUA
 AAUUGAGCCUAAAGAAGAAAUUCUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUA
 AAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAG
 UGGACAAUCGACGGAUAACAGCAUAUCUAG

U1A responsive Circular EGFP variant4 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, U1A binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUAACAACUAAUCG
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUCAGAGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCAUGGGACGCU
CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUCCUAAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUAACCAUAUAGCUAUUGGA
UUGGCGACAGCAUUGUACCCAGAGUCUGUCCCCAGACAUUGCACCUGGCGCUGUCA

UCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUG
 AAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccguga
 gcaagggcgaggagcuguucaccgggguggugcccuccuggucgagcuggacggcgacguaaacggccaca
 aguucagcguugccggcgagggcgagggcgauGCCaccuacggcaagcugaccugaaguucaucugcaccac
 cggcaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuucagccguac
 cccgaccacaugaagcagcacgacuucuuaaguccgccaugcccgaaggcuacguccaggagcgcaccaucu
 ucuuaaggagcagcggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccuggugaaccgcau
 cgagcugaagggcaucgacuuaaggaggacggcaacaucggggcacaagcuggaguacaacuacaacagc
 cacaacgucuauaaucauggccgacaagcagaagaacggcaucaaggugaacuuaagaucggccacaacaucg
 aggacggcagcguagcagcucggcaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcc
 cgacaaccacuaccugagcaccaguccgcccugagcaaagaccccaacgagaagcgcgaucaaugguccugc
 uggaguucgugaccgcccggggaucacucucggcauggacgagcuguacaagagaucauauugcaucucg
 agugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucuccuugcaccuguaccu
 cuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACG
 GACUUAAAUAUUGAGCCUUAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAAACUCAGG
 GAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGU
 AAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

MS2CP responsive A-cap Linear EGFP variant4

Group I intron, CVB3 IRES, EGFP, MS2CP binding motif

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCC
 GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACAC
 CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCU
 AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGU
 AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
 CAAGCACUUCUGUUAACCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
 AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
 GAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUC
 CACGGGCGACCGUGGCGGUGGCUUGGCGGCCUGCCAUUGGGGAAACCCAUUGG
 ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
 CCCUGAAUGCGGCUAAUCCUACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
 GUCGUAAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGUGUCCGUGUUUCAUUUU
 AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGCAGGUGAGGAUCACCCAUCUGCCA
 CGAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUUGGAUCGUUACCAUAUAGCUAUUG
 GAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCAC
 UUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggga

uccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccac
aaguucagcguguccggcgaggcgaggcggaugccaccuacggcaagcugaccugaaguucaucugcaccaccggc
aagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuucagccgcuaccccgaccacau
gaagcagcacgacuucucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucucaaggacgacggc
aacuacaagaccgcgcccaggugaaguucgagggcgacaccuggugaaccgcaucgagcugaagggcaucgacuuc
aaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaag
cagaagaacggcaucaaggugaacucaagaauccgccacaacaucgaggacggcagcgugcagcucgcccgaccacuacc
agcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaa
agaccccaacgagaagcgcgaucauagguccugcuggaguucgugaccgccgcccgggaucacucucggcauggacga
gcuguacaagagaucauauagcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuuc
uucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUUAUGCGUUAC
CGGCGAGACGCUACGGACUUAAA
AAA
AAAAAAAAAAAAAAAAAAAA

MS2CP responsive A-cap Linear EGFP variant5

Group I intron, CVB3 IRES, EGFP, MS2CP binding motif

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCUGAAUUGCGGCUAUUCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGAGCAGGUGAGGAUCACCCAUCUG
CCACGAGCGAGGUGAGGAUCACCCAUCUCGUCUGUGUUCUCCUUGUUGGUUUUAUA
CCACUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAa
ugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacg
gccacaaguucagcguguccggcgaggcgaggcggaugccaccuacggcaagcugaccugaaguucaucugcaccac
cggcaagcugcccugcccuggcccaccucgugaccaccucgaccuacggcgugcagugcuucagccgcuaccccgacc

acaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcgaccauucuucucaaggacga
cggcaacuacaagacccgcgccgaggugaaguucgagggcgacaccuggugaaccgcaucgagcugaagggcaucga
cuucaaggaggacggcaacaucuggggcacaagcuggaguuacaacuacaacagccacaacgucuaaucauggccga
caagcagaagaacggcaucaaggugaacucaagaucgcccacaacaucgaggacggcagcgugcagcucgcccagca
cuaccagcagaacacccccaucggcgacggccccgugcugcugcccagacaaccacuaccugagcaccaguccgcccuga
gcaaagaccccaacgagaagcgcgaucaauggucugcuggaguucgugaccgcccgggaucacucucggcaugg
acgagcuguacaagagaucucauugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcc
cuucuucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGU
UACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AAAAAAAAAAAAAAAAAAAA

MS2CP responsive A-cap Linear EGFP variant6

Group I intron, CVB3 IRES, EGFP, MS2CP binding motif

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAUAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGUCACAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAUUCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUACCCUUGUUGGGUUUAUACCACU
AGCUUGAAAGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAAAGAGCAG
GUGAGGAUACCCAUCUGCCACGAGCGAGGUGAGGAUACCCAUCUCGUCGUGUUCa
ugggauccgugagcaagggcgaggagcuguucacggggguggugcccauccuggucgagcuggacggcgacguaaacg
gccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccucgaagucaucugcaccac
cggcaagcugcccugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgacc
acaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcgaccauucuucucaaggacga
cggcaacuacaagacccgcgccgaggugaaguucgagggcgacaccuggugaaccgcaucgagcugaagggcaucga
cuucaaggaggacggcaacaucuggggcacaagcuggaguuacaacuacaacagccacaacgucuaaucauggccga

caagcagaagaacggcaucaaggugaacucaagaucgccacaacaucgaggacggcagcgugcagcucgcccagcca
 cuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccuga
 gcaaagaccccaacgagaagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucggcaugg
 acgagcuguaacaagagaucucauugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcc
 cuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGU
 UACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAA
 AAAAAAAAAAAAAAAAAAAAA

U1A responsive A-cap Linear EGFP variant4

Group I intron, CVB3 IRES, EGFP, U1A binding motif

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUAAGUCCCUCCACCCCCACGCC
 GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACAC
 CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCU
 AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGU
 AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
 CAAGCACUUCUGUUACCCCGGACUGAGUAUCAUAGACUGCUCACGCGGUUGAAGGAG
 AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
 GAGUGUUUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
 CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGAAACCCAUGGG
 ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
 CCCUGAAUUGCGGCUAAUCCUACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
 GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
 AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUUAUAGCUAUUGGA
 UUGGCGACAGCAUUGUACCCAGAGUCUGUCCCAGACAUUGCACCUGGCGCUGUCCAU
 CCGGUGACUAAUAGAGCUAUUAUUAUACCCUUGUUGGGUUUAUACCACUUAAGCUUGAA
 AGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagg
 gcgaggagcuguuacccgggguggugcccauccugugcagcuggacggcgacguaaacggccacaaguucagcgugu
 ccggcgagggcgagggcgauccaccuacggcaagcugaccugaagucaucugcaccaccggcaagcugcccugcc
 cuggcccaccucugugaccaccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagac
 uucuucaaguccgcaugcccgaaggcuacguccaggagcgcaccaucuuucaaaggacgacggcaacuacaagacc
 gcgccgagggugaaguucgagggcgacaccugguagaaccgcaucgagcugaagggaucgacuuaaggaggacggca
 acauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaauaggccgacaagcagaagaacggca
 ucaaggugaacuuaagaucggccacaacaucgaggacggcagcgugcagcucgcccaccacuaccagcagaaccccc
 caucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaagaccccaacgag
 aagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguaacaagaga

ucucauauagcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucccuugca
ccuguaccucugugucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACG
CUACGGACUUAA
AA
AAAAA

U1A responsive A-cap Linear EGFP variant5

Group I intron, CVB3 IRES, EGFP, U1A binding motif

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACAC
CGGUCGCCACC UAAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGUCACGACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGACAGCAUUGUACCCAGAGUCUGUC
CCCAGACAUUGCACCUGGCGCUGUCUCCCUUUGUUGGUUUUAUACCACUUAAGCUUGAA
AGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAAuggggaucgugagcaagg
gcgaggagcuguuacccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcgugu
ccggcgaggggcgagggcgauccaccuacggcaagcugaccugagaugucaucugcaccaccgggaagcugcccgugcc
cuggccaccucugugaccaccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagcagc
uucuucaaguccgcaugccgaaggcuacguccaggagcgcaccauucuucaaggagcagcggaacuacaagacc
gcgccgaggugaaguucgagggcgacaccugguagaaccgcaucgagcugaaggcgcaucgacuuaaggaggacggca
acaucuggggcacaagcuggaguacaacuacaacagccacaacgucuaaucauggccgacaagcagaagaacggca
ucaaggugaacuuaagaucggccacaacaucgaggacggcagcgugcagcucgcccaccacuaccagcagaacacccc
caucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaagaccccaacgag
aagcgcgaucaaugguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagaga
ucucauauagcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucccuugca
ccuguaccucugugucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACG
CUACGGACUUAA

AA
AAAAA

U1A responsive A-cap Linear EGFP variant6

Group I intron, CVB3 IRES, EGFP, U1A binding motif

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACAC
CGGUCGCCACC

UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGU
AUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCUCCCCAACUGUAACUUAGAAGUA
ACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUG
UUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUU
AUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGU
CAGCACUACCCAGUGUAGAUCAAGGUCGAUGAGUCACCGCAUUCCCACGGGCGACCG
UGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCAUGGGACGCUCUAAUACA
GACAUUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGC
UAAUCCUAAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCA
ACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAUCCUUAUACUGG
CUGCUUAUGGUGACAAUUGAGAGAUCGUUAACCAUAUAGCUAUUGGAUUGGCCAUCCGG
UGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUUAAGCUUGAAAGAG
GUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAAGACAGCAUUGUACCCAGA
GUCUGUCCCGAGACAUUGCACCUGGCGCUGUCaugggauccgugagcaagggcgaggagcuguuac
cggggugggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgaggcgaggg
cgaugccaccuacggcaagcugaccugaagucaucugcaccaccggcaagcugcccuguccugggccaccucgug
accaccugaccuacggcgugcagugcuucagccguaccccgaccacaugaagcagcagcagcuucuuaaguccgcca
ugcccgaaaggcuacguccaggagcgcaccaucucuuaaggacgacggcaacuacaagaccgcgccgaggugaagu
ucgagggcgacaccuggugaaccgcaucgagcugaagggaucgacuuaaggaggacggcaacaucuggggcaca
agcuggaguaacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuua
agaucggccacaacaucgaggacggcagcgugcagcucgcccaccacuaccagcagaacacccccaucggcgacggccc
cgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaaaagacccaacgagaagcgcgaucaaug
guccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucucauauugcaucuc
gagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucccuugcaccuguaccucuuggu
cuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUAACCGGCGAGACGCUACGGACUUA
AA
AA

MS2CP responsive Circular EGFP +pA variant4 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, MS2CP binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUUCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGCAGGUGAGGAUACCCAUUCGCCAC
GAGCGAGGUGAGGAUACCCAUUCGCUUGUGUUCGAUCGUUACCAUAUAGCUAUUG
GAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACC
ACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAa
ugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacg
uaaacggccacaaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccugagaugu
caucugcaccaccggcaagcugcccugcccuggcccaccucugaccaccugaccuacggcgugcagugc
uucagccgcuaccccgaccacaugaagcagcagcagcagcagcagcagcagcagcagcagcagcagcagc
agcgaccacucucucuaagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagc
ggugaaccgcaucgagcugaagggcaucgacucuaagcagcagcagcagcagcagcagcagcagcagc
aacuaacaacagccacaacgucuaaucaugggcgacaagcagaagaacggcaucaaggugaacucuaagauc
gccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggcccc
gugcugcugcccgacaaccacuaccugagcaccaguccgccugagcaagaccccaacgagaagcgcgau
acaugguccugcuggaguucgugaccgccgcccgggaucacucucggcauggagcagcuguaacaagagauc
auaugcaucucgagugauagucagaccuucugcggggcuugccuucuggccaugcccuucucucuccuu
gcaccuguaccucugugucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AAAGGCUAUUAUGCGUUACCG
GCGAGACGCUACGGACUUAUUUAUUGAGCCUUAAGAAGAAUUCUUUAAGUGGAUGC

UCUCAAAACUCAGGGAAACC UAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGA
AGUAGUAAUUAGUAAG ACCAGUGGACAAUCGACGGAUAAACAGCAUAUCUAGACACAGGA
AACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUC
CCCGGGUACCGAGCUCGAAUU

U1A responsive Circular EGFP variant5 (**bold: circularized**)

Group I intron, CVB3 IRES, EGFP, U1A binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC **AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAAGGUCGAUGAGUCACCGCAUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCGGCCCCU
GAAUGCGGCUAAUCCUAAUCUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUAACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGACAGCAUUGUACCCAGAGUCUG
UCCCCAGACAUUGCACCUGGCGCUGUCUCCCUUUGUUGGGUUUAUACCACUUAGCUU
GAAAGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAA **augggauccgug**
agcaagggcgaggagcuguuacacggggguggugcccauccuggucgagcuggacggcgacguaaacggccac
aaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccucgaagucaucugcacca
ccggcaagcugcccuguccugcccaccucgugaccaccugaccuacggcgugcagugcuucagccgcua
ccccgaccacaugaagcagcagcagcuuucuaguccgccaugcccgaaggcuacguccaggagcgaccac
uucuucaaggacgacggcaacuacaagacccgcgcccaggguagauguaggggcgacaccugggugaaccgca
ucgagcugaagggcaucgacucaaggaggacggcaacuuccggggcacaagcuggaguguacaacuacaag
ccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacucaagaucggccacaacauc
gaggacggcgagcugcagcucgcccaccacuaccagcagaacacccccaucggcgacggccccgugcugcugc
ccgacaaccacuaccugagcaccaguccgcccugagcaagaccccaacgagaagcgcgaucaaugguccug
cuggaguucgugaccgcccgggaucacucucggcauggacgagcuguacaagagaucauauugcaucuc

gagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucccuugcaccuguacc
ucuuggucuuu**GAAUAAAGCCUGAGUAGGGGCUAUUUAUGCGUUACCGGCGAGACGCU****AC**
GGACUUAAUAAUUGAGCCUAAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAAACUCAG
GGAAACCUAAAUCUAGUUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUAG
U**AAG**ACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGAC
CAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCG
AGCUCGAAUU

U1A responsive Circular EGFP +pA variant5 (**bold: circularized**)

Group I intron, CVB3 IRES, **EGFP**, U1A binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGU**UGA**
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUACCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGACAGCAUUGUACCCAGAGUCUG
UCCCCAGACAUUGCACCUUGGCGCUGUCUCCCUUGUUGGGUUUAUACCACUUAGCUU
GAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA**augggauccgug**
agcaagggcgaggagcuguuacccgggguggugcccauccuggucgagcuggacggcgacguaaacggccac
aaguucagcguguccggcgagggcgagggcgauGCCaccuacggcaagcugaccucgaagucaucugcacca
ccggcaagcugcccuguccugcccaccucgugaccaccucgaccuacggcgugcagugcuucagccgcua
ccccgaccacaugaagcagcagcagcuucucaaguccgccaugcccgaaggcuacguccaggagcgaccauc
uucuucaaggacgacggcaacuacaagaccgcgcccaggguagauguaggggcgacaccucggugaaccgca
ucgagcugaagggcaucgacucaaggaggacggcaacaucggggcacaagcuggaguacaacuacaacag
ccacaacgucuauaaucauggccgacaagcagaagaacggcaucaaggugaacucaagauccgccacaac

gaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugc
 ccgacaaccacuaccugagcaccaguccgccugagcaagaccccaacgagaagcgcgaucaaugguccug
 cuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguaacaagagaucauauugcaucuc
 gagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucuccuugcaccuguacc
 ucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGC
 UACGGACUUAUUAAUUGAGCCUUAAGAAGAAUUCUUUAAGUGGAUGCUCUCAACU
 CAGGGAAACCUAUUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAU
 UAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

Cap-MS2CP

MS2CP

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACACCGGUcgccaccaugGC
 UUCUAACUUUACUCAGUUCGUUCUCGUCGACAAUGGCGGAACUGGCGACGUGACUGUC
 GCCCCAAGCAACUUCGCUAACGGGGUCGCUGAAUGGAUCAGCUCUAACUCGCGAUCAC
 AGGCUUACAAAGUAACCUGUAGCGUUCGUCAGAGCUCUGCGCAGAAUCGCAAUACACC
 AUCAAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUUAUUAAUUAUGGAACUAACCAU
 UCCAAUUUUCGCCACGAAUCCGACUGCGAGCUUAUUGUUAAGGCAAUGCAAGGUCUCC
 UAAAAGAUGGAAACCCGAUUCUCCUCGGCCAUCGCGGCCAACUCCGGCAUCUACUGAucua
 gaccuucugcggggcuugccuucuggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAA
 GCCUGAGUAGGAAA
 AA
 AAAAAA

Cap-U1A

U1A

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACACCGGUcgccaccAUGG
 cgGCAGUUCGCGAGACCCGCCCUAACACACUAUUUAUAUCAACAACCUCAAUGAGAAGA
 UCAAGAAGGAUGAGCUAAAAAGUCCCUGUACGCCAUCUUCUCCAGUUUGGCCAGAUC
 CUGGAUAUCCUGGUUAUCACGGAGCCUGAAGAUGAGGGGCCAGGCCUUUGUCAUCUUA
 AGGAGGUCAGCAGCGCCACCAACGCCUGCGCUCCAUGCAGGGUUUCCCUUUCUAUGA
 CAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCAGAUUAUUGCCAAGAUGAAAG
 GCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGAGGAAGCCCAAGAGCCAGGAGAC
 CCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGGAGCCACCCCGUGGUGGGGGCUGU
 CCAGGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUCAGGCGCCCGCAUUAUGCACCAC

AUGCCGGGCCAGCCGCCCACAUGCCGCCCCUGGUAUGAUCCCCCGCCAGGCCUUG
CACCUGGCCAGAUCCACCAGGGGCCAUGCCCCCGCAGCAGCUUAUGCCAGGACAGAU
GCCCCUGCCCAGCCUCUUUCUGAGAAUCCACCGAAUCACAUCUUGUCCUCACCAACC
UGCCAGAGGAGACCAACGAGCUC AUGCUGUCCAUGCUUUUCAUAGUCCUGGCCU
CAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUCGCCUUCGUGGAGUUUGACAAU
GAGGUACAGGCAGGGGCAGCUCGCGAUGCCCUGCAGGGCUUUAAGAUCACGCAGAAC
ACGCCAUGAAGAUCUCCUUUGCCAAGAAGUAGucuaagaccuucugcggggcuugccuucuggccaugc
ccuucucucuccuugcaccuguaccucuuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAA
AA
AA

3'T302a-5p Circular MS2CP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, miR-302a-5p target site, MS2CP

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUAACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUC AAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGUAACUUAAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUCAUUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU CGUUAACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAaugG
CUUCUAACUUUACUCAGUUCGUUCUGUCGACAAUGGCGGAACUGGCGACGUGACUG
UCGCCCCAAGCAACUUCGCUAACGGGGUCGCGUAAUGGAUCAGCUCU AACUCGCGAU
CACAGGCUUACAAAGUAACCGUAGCGUUCGUCAGAGCUCUGCGCAGAAUCGCAAAU
ACACCAUCAAAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUUAUUAAUAUGGAAC

UAACCAUUCCAAUUUUCGCCACGAAUUCGACUGCGAGCUUAUUGUUAAGGCAAUGCA
 AGGUCUCCUAAAAGAUGGAAACCCGAUUCCCUCGGCCAUCGCGGCCAACUCCGGCAU
 CUACUGAAGCAAGUACAUCCACGUUAAGUucuagaccuucugcggggcuugccuucuggccau
 gcccuucucucuccuugcaccguuaccucuuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAA
 AA
 AAGGC
 UAUUAUGCGUUACCGGCGAGACGCUACGGACUUAUUAAUUGAGCCUUAAGAAGAAA
 UUCUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAUUAUAGUUAUAGACAAGGCAA
 UCCUGAGCCAAGCCGAAGUAGUAAUAGUAAGACCAGUGGACAAUCGACGGAUAACAGC
 AUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGG
 UCGACUCUAGAGGAUC

3'T302a-5p Circular U1A +pA (**bold: circularized**)

Group I intron, CVB3 IRES, miR-302a-5p target site, U1A

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUAACAACUAAUCG
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUACGCUAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
 CCUUAACCGGUCGUGUGGGUUAAGUCCCUCCACCCACGCGGAAACGCAAUAGC
 CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
 UUAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
 UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGUAACUAGAAG
 UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
 CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
 CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
 UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
 GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCAUUGGGGAAACCCAUGGGACGCU
 CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
 GAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
 GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUCAUUUUUAU
 UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUUGUUAACCAUAUAGCUAUUGGA
 UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUUAUACCCUUGUUGGGUUUAUACCACU
 UAGCUUGAAAGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAAUGG
 cgGCAGUUCCCGAGACCCGCCCUAACCACACUAUUUAUUAACAACAACCUCAAUGAGAA
 GAUCAAGAAGGAUGAGCUAAAAAGUCCUGUACGCCAUCUUCUCCAGUUUGGCCA
 GAUCCUGGAUAUCCUGGUUAUCACGGAGCCUGAAGAUGAGGGGCCAGGCCUUUGUCAU
 CUUCAAGGAGGUCAGCAGCGCCACCAACGCCUUGCGCUCCAUGCAGGGUUUCCCUU

CUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCAGAUUAUCAUUGCCAAG
AUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGAGGAAGCCCAAGAGC
CAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGAGCCACCCCGUGGUG
GGGGCUGUCCAGGGGCCUGUCCGGGCAUGCCGCCGAUGACUCAGGCGCCCCGCAUU
AUGCACCACAUGCCGGGCCAGCCGCCCUACAUGCCGCCCCUGGUAUGAUCCCCCG
CCAGGCCUUGCACCUGGCCAGAUCCCACCAGGGGCCAUGCCCCCGCAGCAGCUUAUG
CCAGGACAGAUGCCCCUGCCCAGCCUCUUUCUGAGAAUCCACCGAAUCACAUCUUGU
UCCUCACCAACCUGCCAGAGGAGACCAACGAGCUCUAUGCUGUCCAUGCUIUUCAAUCA
GUUCCUGGCUUCAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUCGCCUUCGU
GGAGUUUGACAAUGAGGUACAGGCAGGGGCAGCUCGCGAUGCCUGCAGGGCUUUA
GAUCACGCAGAACACGCCAUGAAGAUCUCCUUGCCAAGAAGUAGAGCAAGUACAU
CCACGUUUAAGUUCUAGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUCUCCUUGCACC
UGUACCUCUUGGUCUUUGAAUAAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AAGGCUAUUAUGCGUUACCGGCGA
GACGCUACGGACUUAUAAUUAUUGAGCCUUAAGAAGAAUUCUUUAAGUGGAUGCUCUC
AAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUA
GUAAUUAAGUAGACAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACA
GCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUC

EGFP linear switch (MS2CP)

EGFP, MS2CP binding motif

GGUCAGAUCCGCUAGCGGAUCCGGGAGCAGGUGAGGAUCACCCAUCUGCCACGAGCGA
GGUGAGGAUCACCCAUCUCGCGUCGUGUCCCCACCGGUCGCCaccauggggaucgugagcaagggc
gaggagcugucaccgggguggugcccaccugugcagcugggcggcgacguaaacggccacaaguucagcugucc
ggcgaggggcgaggggcgaugccaccuacggcaagcugaccucgaaguucacugcaccaccggcaagcugcccugccc
ggcccaccucgugaccaccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcagcagcuu
cuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuuucaaggacgacggcaacuacaagaccggc
gccgaggugaaguucgagggcgacaccuggugaaccgcaucgagcugaagggcaucgacuucaggaggacggcaac
auccuggggcacaagcuggaguacaacuacaacagccacaacgucuaaucauggccgacaagcagaagaacggcauc
aaggugaacuucagaauccgccacaacaucgaggacggcagcgugcagcucgcccaccacuaccagcagaacaccccc
ucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgcccugagcaagacccccaacgagaa
gcgcgaucacaugguccugcuggaguucgugaccgcccgggaucacucucggcauggacgagcuguaacaagagauc
ucauauagcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucucucuccuugcacc
uguaccucuuggucuuuGAAUAAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA

AAAAAAAAAAAAAAAAAAAAAAAAAAAA

EGFP linear switch (U1A)

EGFP, U1A binding motif

GACAGCAUUGUACCCAGAGUCUGUCCCCAGACAUUGCACCUGGCGCUGUC CGCAGAUCC
GAGAAGAAGGCGAAUUAAGAGAGAAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUgcc
accauggggauccgugagcaagggcgaggagcuguuacccgggguggugcccauccuggucgagcuggacggcgacgua
aacggccacaaguucagcguguccggcgaggcgaggcgaguccaccuacggcaagcugaccugaaguucaucugc
accaccggcaagcugcccugcccuggcccaccucgugaccaccugaccuacggcgugcagugcuucagccgcuacc
cgaccacaugaagcagcagcagcuucuaaguccgccaugcccgaaggcuacguccaggagcgaccacuuuuaag
gacgacggcaacuacaagaccgcgcccaggugaaguucgagggcgacaccuggugaaccgcaucgagcugaagggc
aucgacuuaaggaggagcggaacaucuggggcacaagcuggaguacaacuacaacagccacaacgucuauaaucaug
gccgacaagcagaagaacggcaucaaggugaacuuaagaucggccacaacaucgaggacggcagcgugcagcucgcc
gaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcaccaguccgc
ccugagcaaagacccaacgagaagcgcgaucaauguccugcuggaguucgugaccgccggggaucaucucugggc
auggacgagcuguacaagagaucauauugcaucucgagugauagucuagaccuucugcggggcuugccuucuggcca
ugcccuucucucuccuugcaccguuaccucuuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAA
AA
AA

MS2CP responsive Circular MetLuc2 +pA variant4 (**bold: circularized**)

Group I intron, CVB3 IRES, MetLuc2, MS2CP binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC AACAAUAGAUGACUUAACAACUAAUCCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAGGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCCCAACUGUAACUUAAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC

GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUAU
 UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGCAGGUGAGGAUACCCAUUCGCCAC
 GAGCGAGGUGAGGAUACCCAUUCGCGUGUGUUGAUCGUUACCAUAUAGCUAUUG
 GAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACC
 ACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAa
 uggacaucaaggugguguucaccugguugucagcgccugguugcaggccaagagcaccgaguucgacccca
 acaucgacaucguggggccuggaaggcaaguucggcaucaccaaccuggaaaccgaccguucaccaucuggga
 gaccauggaagugaugaucaaggccgacaucgcccagaccgggcccagcaacuucguggccaccgagacc
 gacgccaaccggggcaagaugcccggcaagaagcugccccugggccgucaucauggaauggaagccaacgccu
 ucaaggccggcugcaccggggcugccugaucugccugagcaagaucagcaccgccaagaugaaggugua
 cauccccggcaggugccacgacuacggcgggcacaagaaaaccggccaggccggcaucguggggcgccaucgug
 gacaucccgagaucaagcgccucaaagaauggcccccauggaacaguucaucgcccaggugggacagaugcg
 ccagcugcaccaccggcugccugaaggggccuggccaacgugaagugcagcgagcugcugaagaaguggcugcc
 cgaccgugcgccagcuucgcccacaagaucagaaagaggugcacaacaucaggggcauggccggcgacagg
 ugaucuagaccuucugcggggcuugccuucuggccaugcccuucucucccuugcaccuguaccucuuagg
 ucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUAACGGCGAGACGCU**ACGG**
ACUUAAAUAUUGAGCCUUAAGAAGAAAUUCUUAAGUGGAUGCUCUCAAACUCAGGG
 AAACCUAAAUUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAAGUA
 AGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCA
 UGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAG
 CUCGAAUU

U1A responsive Circular MetLuc2 +pA variant5 (**bold: circularized**)

Group I intron, CVB3 IRES, MetLuc2, U1A binding motif

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC**AACAAUAGAUGACUUAACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCACGCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUAUACCCCUCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU

UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
 GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
 CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
 GAAUGCGGCUAUACCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
 GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAU
 UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUUAUAGCUAUUGGA
 UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGACAGCAUUGUACCCAGAGUCUG
 UCCCCAGACAUUGCACCUGGCGCUGUCUCCCUUUGUUGGGUUUAUACCACUUAAGCUU
 GAAAGAGGUUAAAACAUUACAAUUAUUGUUAAGUUGAAUACAGCAAAugggacaucaag
 gugguguuacaccugguguuacagcgcccuggugcaggccaagagcaccgaguucgacccaacaucgacaucg
 ugggccuggaagggaaguucggcaucaccaaccuggaaaccgaccguuaccaucugggagaccauggaagu
 gaugaucaaggccgacaucgcccacacgaccggggccagcaacuucguggccaccgagaccgacgccaaccgg
 ggcaagaugcccggcaagaagcugccccuggccgucaucauggaaauggaagccaacgccuuaaggccggcu
 gcacccggggcugccggaucugccugagcaagaucagugcaccgccaagaugaagguguacauccccggcag
 gugccacgacuacggcgccgacaagaaaaccggccaggccggcaucguggggcgccaucguggacaucacccgag
 aucagcgccuuaaagaaauggcccccauggaacaguucaucgcccagguggacagaugcgccagcugcacca
 ccggcugccugaagggccuggccaacgugaagugcagcgagcugcugaagaaguggcugcccgaccgugcg
 ccagcuucgcccagacaagaucagaaagguggcacaacaucaggggcauggccggcgacaggugaucuagacc
 uucugcgggggcuugccuucuggccaugcccuucucucucccuugcaccuguaccucuuggucuuuGAAUA
 AAGCCUGAGUAGGAA
 AA
 AAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUAACCGGCGAGACGCUACGGACUUAUAUA
 AUUGAGCCUUAAGAAGAAUUCUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAA
 UCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAUAGUAAGACCAGUG
 GACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGC
 CAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3'T21-5p Circular MS2CP +pA (**bold: circularized**)

Group I intron, CVB3 IRES, miR-21-5p target site, MS2CP

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUAACAACUAAUCG
 GAAGGUGCAGAGACUCGACGGGAGCUACCCUAAAGCUAAGACGAGGGUAAAGAGAGAG
 UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGUUGA
 CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
 CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
 UUAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
 UAUCACGGUACCUUUGUGCGCCUGUUUAUACCCCCUCCCCAACUGUAACUAGAAG

UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
 CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
 CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
 UUCGCUCAGCACUACCCAGUGUAGAUCAAGGUCGAUGAGUCACCGCAUUCCCCACGG
 GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCAUGGGACGCU
 CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCGGCCCCU
 GAAUGCGGCUAUUCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
 GUAACGGGCAACUCUGCAGCGGAACCGACUACUUGGGUGUCCGUGUUUCAUUUUUAU
 UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
 UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
 UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA **augG**
CUUCUAACUUUACUCAGUUCGUUCUGCUGCACA AUGCGGAACUGGCGACGUGACUG
UCGCCCCAAGCAACUUCGCUAACGGGGUCGCUGAAUGGAUCAGCUCU AACUCGCGAU
CACAGGCUUACAAAGUAACCGUAGCGUUCGUCAGAGCUCUGCGCAGAAUCGCAAAU
ACACCAUCAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUACUUAUAUAUGGAAC
UAACCAUUCCAAUUUUUCGCCACGAAUUCGACUGCGAGCUUAUUGUUAAGGCAAUGCA
AGGUCUCCUAAAAGAUGGAAACCCGAU UCCCUUGGCCAU CGCGGCCAACUCCGGCAU
CUACUGAUC AACAU CAGUCUGAU AAGCU ucuagaccuucugcggggcuugccuucuggccaugc
 ccuucucucuccuugcaccuguaccucuuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAA
 AA
 AAAGGCUA
 UUAUGCGUUAACCGGCGAGACGCU **ACGGACU** UAAAUAUUGAGCCUUAAGAAGAAAU
 CUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUC
 CUGAGCCAAGCCGAAGUAGUAAUAGUAAG ACCAGUGGACAAUCGACGGAUAACAGCAU
 AUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUC
 GACUCUAGAGGAUC

3'T21-5p Circular U1A +pA (**bold: circularized**)

Group I intron, CVB3 IRES, miR-21-5p target site, U1A

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUC **AACAAUAGAUGACUUAACAACUAAUCG**
GAAGGUGCAGAGACUCGACGGGAGCUACCCU AACGUC AAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUGAAAAUCCGU UGA
CCUUAACGGUCGUGUGGGUUAAGUCCUCCACCCCCACGCCGGAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUAUACCCCCUCCCCAACUGUAACUAGAAG

UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
 CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
 CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
 UUCGCUCAGCACUACCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCGCCACGG
 GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
 CUAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
 GAAUGCGGCUAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
 GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUUAU
 UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
 UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACU
 UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAA**AUGG**
cgGCAGUUCCCGAGACCCGCCCUAACCACACUAUUUAUAUCAACAACCUCAAUGAGAA
 GAUCAAGAAGGAUGAGCUAAAAAAGUCCUGUACGCCAUCUUCUCCAGUUUGGCCA
 GAUCCUGGAUAUCCUGGUUACACGGAGCCUGAAGAUGAGGGGCCAGGCCUUUGUCAU
 CUUCAAGGAGGUCAGCAGCGCCACCAACGCCUGCGCUCCAUGCAGGGUUUCCCUUU
 CUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCAGAUUAUCAUUGCCAAG
 AUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGAGGAAGCCCAAGAGC
 CAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGGAGCCACCCCGUGGGUG
 GGGGCUGUCCAGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUCAGGCGCCCCGCAUU
 AUGCACCACAUGCCGGGGCCAGCCGCCCUACAUGCCGCCCCUGGUUAUGAUCCCCCG
 CCAGGCCUUGCACCUGGCCAGAUCCCACCAGGGGCCAUGCCCCCGCAGCAGCUUAUG
 CCAGGACAGAUGCCCCUGCCCAGCCUCUUUCUGAGAAUCCACCGAAUCACAUCUUGU
 UCCUCACCAACCUGCCAGAGGAGACCAACGAGCUCAUGCUGUCCAUGCUIIUCAAUCA
 GUUCCUGGCUUCAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUCGCCUUCGU
 GGAGUUUGACAAUGAGGUACAGGCAGGGGCAGCUCGCGAUGCCCUGCAGGGCUUUA
 GAUCACGCAGAACACGCCAUGAAGAUCUCCUUGCCAAGAAGUAG**UCAACAUCAGUC**
UGAUAAGCUAucuagaccuucugcggggcuugccuucugggcaugcccuucucucucccuugcaccugu
 acccuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AA
 AAAGGCUAUUAUGCGUUACCGGCGAGA
 CGCU**ACGGACU**UAAUAAUUGAGCCUUAAGAAGAAAUUCUUAAGUGGAUGCUCUCAA
 ACUCAGGGAAACCUAAAUCUAGUUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGU
 AAUUAAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGC
 UAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUC

MS2CP, miR-302a-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcAGCAAGUAC
AUCCACGUUUUAAGUgccaccaugGCUUCUAACUUUACUCAGUUCGUUCUCGUCGACAAUGG
CGGAACUGGGCGACGUGACUGUCGCCCCAAGCAACUUCGCUAACGGGGUCGCGUGAAUGG
AUCAGCUCUAACUCGCGAUCACAGGCUUACAAAGUAACCUGUAGCGUUCGUCAGAGCUC
UGCGCAGAAUCGCAAAUACACCAUCAAAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUU
ACUUAUUUAUGGAACUAACCAUUCCAAUUUUCGCCACGAAUUCGACUGCGAGCUUAUU
GUUAAGGCAAUGCAAGGUCUCCUAAAAGAUGGAAACCCGAUUCCCUCGGCCAUCGCGG
CCAACUCCGGCAUCUACUGAucuagaccuucugcggggcuugccuucuggccaugccuucucuccuu
gcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAA
AAA
AAA

5'T302a-5p U1A

U1A, miR-302a-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcAGCAAGUAC
AUCCACGUUUUAAGUgccaccAUGGcgGCAGUUCCCGAGACCCGCCCUAACACACUAUUUA
UAUCAACAACCUCAAUGAGAAGAUCAAGAAGGAUGAGCUAAAAAGUCCUGUACGCCA
UCUUCUCCAGUUUGGCCAGAUCCUGGAUAUCCUGGUAUCACGGAGCCUGAAGAUGAG
GGGCCAGGCCUUUGUCAUCUUAAGGAGGUCAGCAGCGCCACCAACGCCUGCGCUC
AUGCAGGGUUUCCCUUUCUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUC
AGAUUAUCAUUGCCAAGAUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAG
AGGAAGCCCAAGAGCCAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGAG
CCACCCCCGUGGUGGGGGCUGUCCAGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUC
AGGCGCCCCGCAUUAUGCACCACAUGCCGGGCCAGCCGCCCUACAUGCCGCCCCCUGG
UAUGAUCCCCCGCCAGGCCUUGCACCUGGCCAGAUCCACCAGGGGCCAUGCCCCCG
CAGCAGCUUAUGCCAGGACAGAUGCCCCCUGCCAGCCUCUUUCUGAGAAUCCACCGAA
UCACAUCUUGUCCUCACCAACCUGCCAGAGGAGACCAACGAGCUCAUGCUGUCCAUGC
UUUUCAAUCAGUUCCUGGCUUCAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAU
CGCCUUCGUGGAGUUUGACAAUGAGGUACAGGCAGGGGCAGCUCGCGAUGCCUGCAG
GGCUUUAAGAUCACGCAGAACAACGCCAUGAAGAUCUCCUUUGCCAAGAAGUAGucuagac
cuucugcggggcuugccuucuggccaugccuucucuccuugcaccuguaccucuuggucuuuGAAUAAAGC
CUGAGUAGGAAA
AAA
AAAA

5'T21-5p MS2CP

MS2CP, miR-21-5p target site

GGGCGAAUUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcUCAACAUCA
GUCUGAUAAAGCUAgccaccaugGCUUCUAACUUUACUCAGUUCGUUCUCGUCGACAAUGGC
GGAACUGGCGACGUGACUGUCGCCCCAAGCAACUUCGCUAACGGGGUCGCUGAAUGGA
UCAGCUCUAACUCGCGAUCACAGGCUUACAAAGUAACCUGUAGCGUUCGUCAGAGCUCU
GCGCAGAAUCGCAAAUACACCAUCAAAGUCGAGGUGCCUAAAAGGCGCAUGGAGGUCUUA
CUUAAAUAUGGAACUAACCAUUCCAAUUUUCGCCACGAAUUCGACUGCGAGCUUAUUG
UUAAGGCAAUGCAAGGUCUCCUAAAAGAUGGAAACCCGAUUCCUCGGCCAUCGCGGCC
AACUCCGGCAUCUACUGAucuagaccuucugcggggcuugccuucuggccaugcccuucucucuccuugca
ccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

5'T21-5p U1A

U1A, miR-21-5p target site

GGGCGAAUUAAGAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGACACCGGUcUCAACAUCA
GUCUGAUAAAGCUAgccaccAUGGcgGCAGUUCCCGAGACCCGCCCUAACACACUAUUUAU
AUCAACAACCUCAAUGAGAAGAUCAAGAAGGAUGAGCUAAAAAGUCCCUGUACGCCAU
CUUCUCCCGAGUUUGGCCAGAUCCUGGAUAUCCUGGUUAUCACGGAGCCUGAAGAUGAGG
GGCCAGGCCUUUGUCAUCUUAAGGAGGUCAGCAGCGCCACCAACGCCCUUGCGCUCCA
UGCAGGGUUUCCCUUUCUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCA
GAUAUCAUUGCCAAGAUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGA
GGAAGCCCAAGAGCCAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGAGC
CACCCCGUGGUGGGGGCUGUCCAGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUCA
GGCGCCCCGCAUUAUGCACCACAUGCCGGGCCAGCCGCCCUACAUGCCGCCCCCUUGGU
AUGAUCCCCCGCCAGGCCUUGCACCUUGGCCAGAUCCACCAGGGGCCAUGCCCCCGC
AGCAGCUUAUGCCAGGACAGAUCCCCCUGCCAGCCUCUUUCUGAGAAUCCACCGAAU
CACAUUCUUGUCCUCACCAACCUGCCAGAGGAGACCAACGAGCUCAUGCUGUCCAUGCU
UUUCAAUAGUUCUCCUGGCUUCAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUC
GCCUUCGUGGAGUUUGACAAUGAGGUACAGGCAGGGGCAGCUCGCGAUGCCCUGCAGG
GCUUUAAGAUCACGCAGAACACGCCAUGAAGAUCUCCUUUGCCAAGAAGUAGucuagacc
uucugcggggcuugccuucuggccaugcccuucucucuccuugcaccuguaccucuuggucuuuGAAUAAAGCC
UGAGUAGGAA
AA
AAA

MetLuc2, MS2CP binding motif

MetLuc2 linear switch (U1A)

MetLuc2, U1A binding motif

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Supplementary References

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