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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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endogenous miRNA activity. We have succeeded in regulating gene expression using miRNA-responsive switches in a cell type-specific manner for various applications, including cell purification derived from human pluripotent stem cells, selective killing of cancer cells, and genome editing of target cells (22–25). Notably, these miRNA-responsive switches did not change the expression level of endogenous target miRNA and global gene expression patterns regulated by miRNAs, indicating that perfect base-pairing between miRNA and its antisense target induces mRNA cleavage through siRNA-like pathways with the RNA-induced silencing complex (RISC) (i.e. miRNA-responsive switches do not function as a miRNA 'sponge') (22,25). Studies on endogenous circRNAs suggested that circRNAs with partially matched miRNA target sites have been reported as potential miRNA sponges or inhibitors (26–28), whereas circRNAs with a near-perfect miRNA target site undergo degradation by sensing miRNA, much like linear mRNAs (29). Therefore, to perform cell-type-specific protein expression from circRNA, we expected that miRNA-responsive switch technology could be applied.

RNA-binding protein (RBP)-responsive mRNA switches also allow the control of protein expression from linear mRNAs in a cell-type-specific manner. In previous studies, protein-responsive switches have been designed to repress cap-dependent translation of linear mRNAs by inserting the protein-binding aptamer sequence at the 5′-UTR (23,30,31). The virus-derived Internal Ribosome Entry Site (IRES) can be used for designing translatable circRNA (2,3), and its underlying translation mechanism and the composition of its initiation factors are similar to those of cap-dependent translation (32). We expected that IRES-dependent translation could be controlled by RBPs, similar to the protein-responsive switches reported previously (23,30,31). So far, endogenous circRNAs have been reported to function as sponges or scaffolds for RBPs (33–36), and synthetic circRNA devices have mimicked these functions, only as RBP regulators or inhibitors (18,37,38). In translatable circRNAs, no devices have been reported in which RBPs regulate the translation of circRNA. Thus, we aimed to investigate the design principles of protein-responsive switches for cap-independent translation of circRNA.

Here we present a design strategy for developing synthetic circRNA devices that control circRNA translation in a cell type-specific manner by detecting target miRNAs and RBPs (Figure 1). We designed miRNA- and RBP-responsive circRNA switches by engineering the UTRs and Coxsackievirus B3 (CVB3) IRES and constructed synthetic circRNA circuits for RNA-only delivery. The designed circRNA circuits showed better expression level persistence than linear mRNA-based circuits.

MATERIALS AND METHODS

Template plasmid construction for circRNA and linear mRNA

All PCRs were performed by PrimeSTAR MAX DNA polymerase (TaKaRa). After the template was digested by DpnI (TOYOBO) at 37°C for 30 min, PCR products were purified by QIAquick PCR Purification Kit (QIAGEN) or Monarch DNA Gel Extraction Kit (New England Biolabs). To prepare template plasmids for RNA, PCR products were cloned into PCR-linearized pUC19 vector by Gibson assembly using NEBuilder HiFi DNA Assembly Master Mix (New England Biolabs) and transformed to DynaCompetent Cells JetGiga *Escherichia coli* DH5α (BioDynamics Laboratory). After culturing *Escherichia coli* at 37°C for 8–16 h with 3 ml LB/Ampicillin (Amp) media, plasmids were purified by NucleoSpin Plasmid EasyPure (TaKaRa). For template plasmids for internal polyA-containing circRNAs, the internal polyA part was inserted and cloned by using synthetic ssDNA has A120 mer with 20 nt overhang for assembling (5′-ttGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCTATTATGCGTTACCGGC-3′). Then, transformed *E. coli* was cultured with 50 ml LB/Amp media, and plasmids were purified by PureYield Plasmid Midiprep System (Promega). All plasmid sequences were checked by the Sanger sequencing method using BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) and 3500xL Genetic Analyzer (Thermo Fisher Scientific).

Synthesis and purification of circRNA and linear mRNA

DNA templates for in vitro transcription (IVT) were amplified from template plasmids using PrimeSTAR MAX DNA polymerase (TaKaRa). After the template plasmid was digested by DpnI (TOYOBO) at 37°C for 30 min, PCR products were purified by QIAquick PCR Purification Kit (QIAGEN). For IVT templates for internal polyA-containing circRNAs, template plasmids were linearized by EcoRI-HF (New England Biolabs) or BamHI-HF (New England Biolabs) and purified by Monarch PCR & DNA Cleanup Kit (New England Biolabs).

Synthesis of mRNAs used the MEGAscript T7 Kit (Thermo Fisher Scientific). For capped linear mRNA synthesis, transcription was performed with 75 mM Anti Reverse Cap Analog (TriLink BioTechnologies) or G(5′)ppp(5′)A RNA Cap Structure Analog (A-cap) (New England Biolabs Japan) in a GTP 4:1 solution. For modified base-containing circRNA and linear mRNA synthesis, pseudouridine-5′-triphosphate (Ψ) and 5-methylcytidine-5′-triphosphate (m5C) or N1-methylpseudouridine-5′-triphosphate (m1Ψ) (TriLink BioTechnologies) were used instead of uridine triphosphate (U) and cytidine triphosphate (C). IVT reaction mixtures were incubated at 37°C for up to 6 h and then mixed with TURBO DNase (Thermo Fisher Scientific), and additionally incubated at 37°C for 30 min to remove the template DNA. The resulting RNAs were purified by the Monarch RNA Cleanup Kit (New England Biolabs). For circRNAs, they were incubated at 55°C with splicing buffer (50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.5, 2 mM GTP) for 30 min and purified again. Purified RNAs were subjected to 4% denaturing polyacrylamide gel electrophoresis (PAGE) (8.3 M urea) and subsequent elution from the gel by overnight incubation at 37°C with 200 rpm shaking in elution buffer (0.3 M

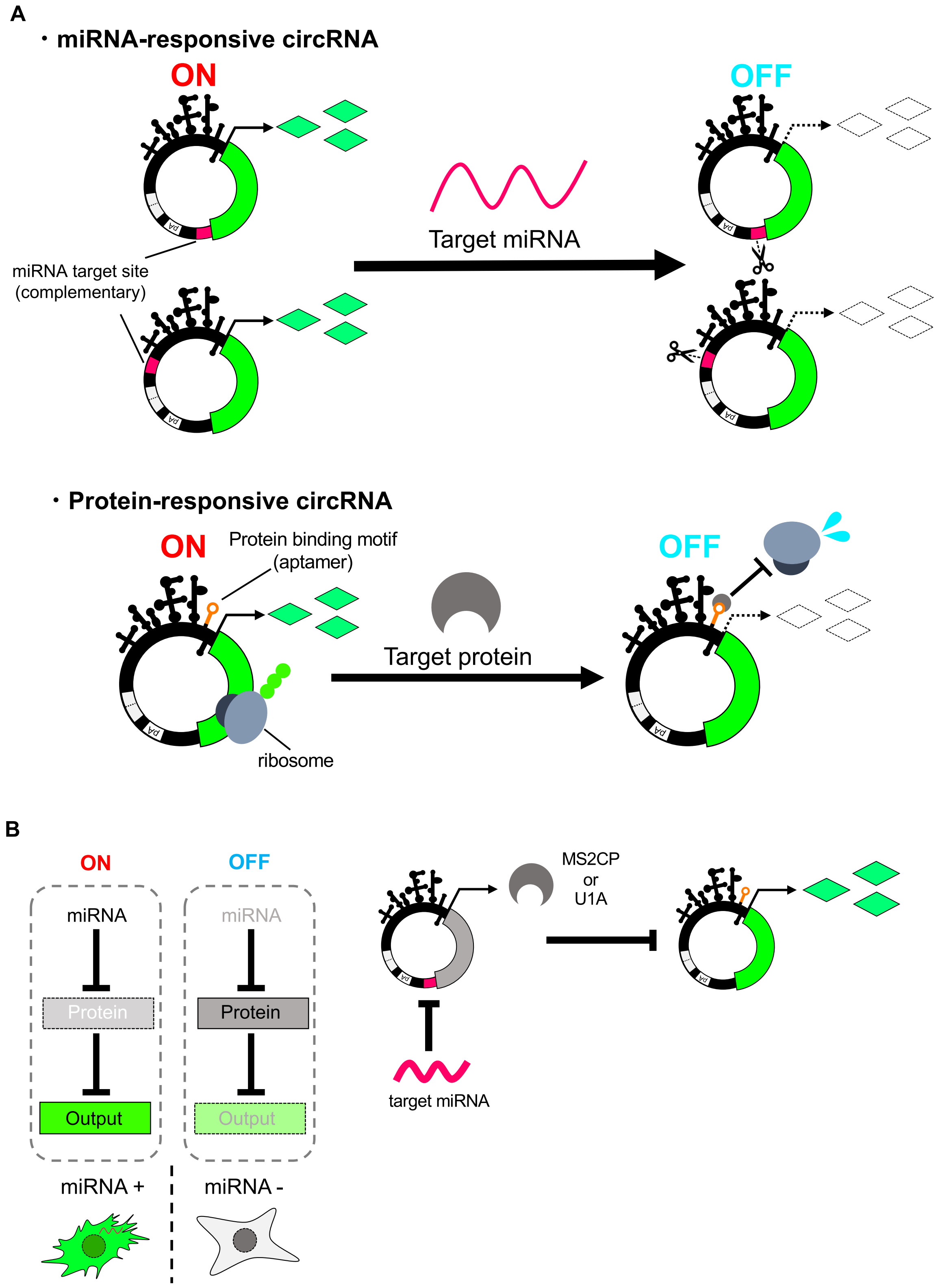

Figure 1. Schematic illustrations of circRNA switches and circuits. (**A**) Design of miRNA or protein-responsive circRNA switch. miRNA-responsive circRNA has the antisense of target miRNA sequence at the UTR. Protein-responsive circRNA has a protein-binding motif in IRES region. In both systems, gene 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 Urtracel-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 AAAAGCTATGCTCTTTTTAGCTCATCCTTCTTG ATCTATAGTGAGTCGTATTAGC-3′, Control-shRNA 5′ -CTGCCTAAGGTTAAGTCGCCCTCGCCTATGC TGCGAGGGCGACTTAACCTTAGGCTATAGTGA 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′- GCCUAAGGUUAAGUCGCCCU CGCAGCAUAGGCGAGGGCGACUUAACCUUA 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% CO_2. 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 expressed 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′-gtagcggctgaagcactgcacg-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 μl 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 μl reaction volume. In both cases, the synthesized cDNA solution was diluted by nuclease-free water with 5-fold dilution. One μl of the diluted-cDNA solution was analyzed by qPCR. The qPCR analysis was performed using THUNDERBIRD Next SYBR qPCR Mix (TOYOBO) with 20 μl 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 ΔCt or ΔΔCt 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 μl 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 μl/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 μl of harvested media was transferred into a Greiner LUMITRA 200 microplate (Greiner), and then 10 μl of 0.5× 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

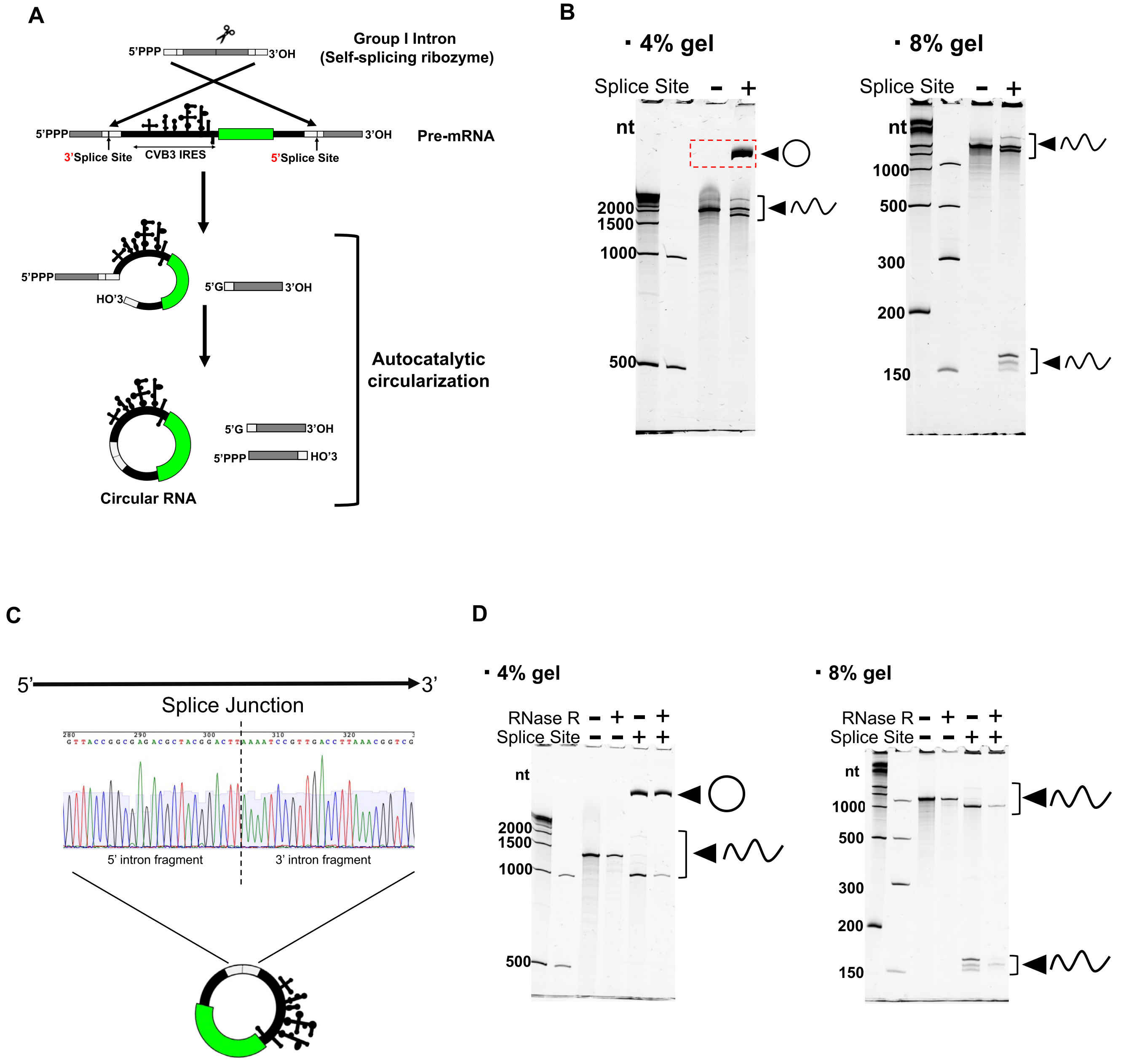

Figure 2. circRNA construction by permuted intron-exon (PIE) splicing. (**A**) Schematic illustration of PIE splicing. (**B**) Denaturing PAGE gel images after *in vitro* transcribed circRNA (ΔpA). DynaMarker RNA High (BioDynamics Laboratory) and Low Range ssRNA Ladder (NEB) were used as molecular weight 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 reporter 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 ΔpA), without IRES (circRNA + pAΔIRES, circRNA ΔpAΔIRES), and three types of linRNAs (Linear EGFP, Cap-EGFP) (Figure 3A).

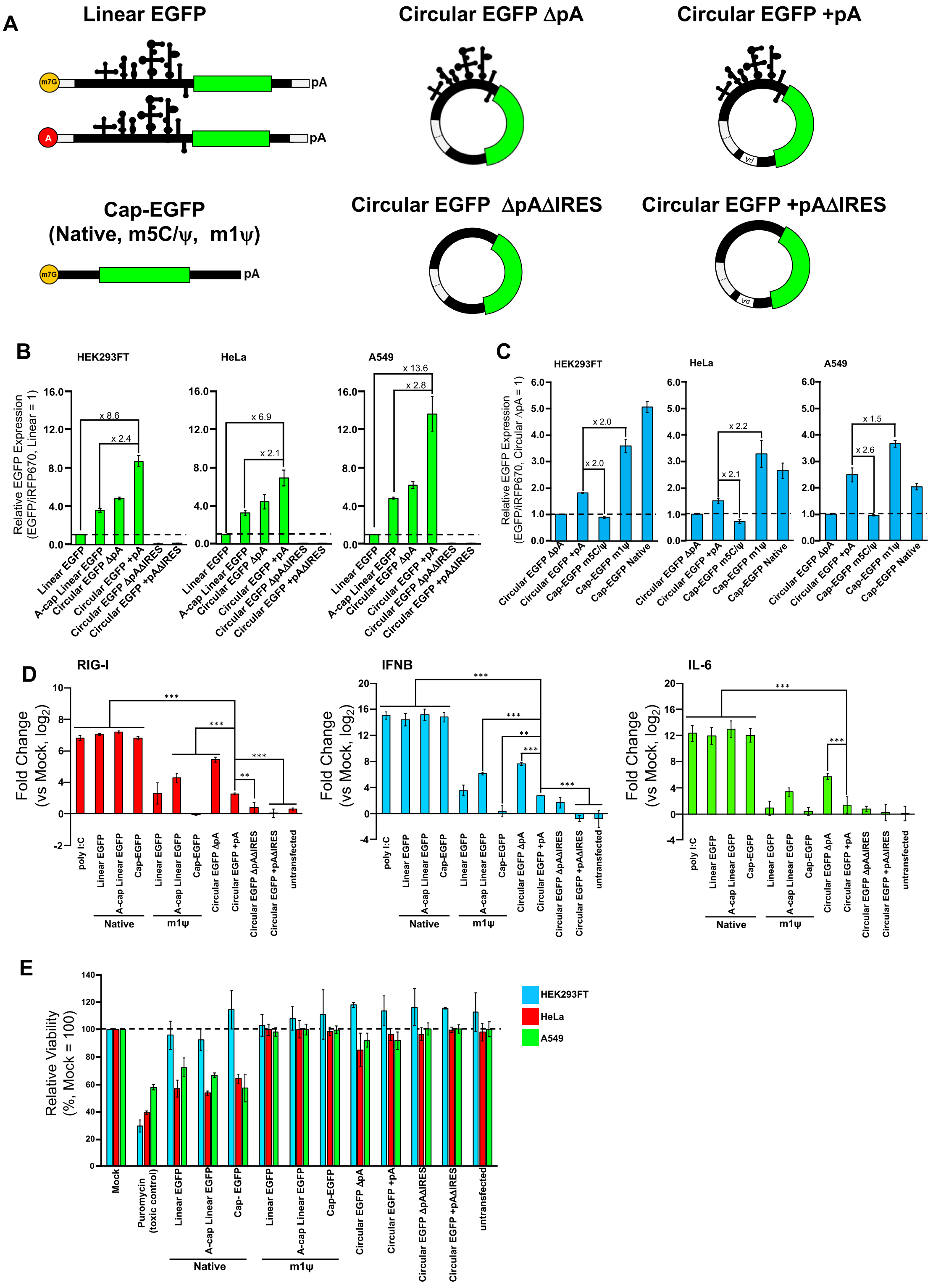

Figure 3. Comparison of reporter expression and immunogenicity. (**A**) Structure illustration of linear mRNAs and circRNAs used in these evaluations. All mRNAs code *EGFP* as a reporter gene. (**B**) Comparison of reporter expression between circRNAs and linear mRNAs with the same sequence component. 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 μg/ml (HEK293FT, HeLa) or 2 μg/ml (A549) puromycin for 1 day before the measurement. All data in this figure are presented as mean ± 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 ΔIRES did not activate translation (Figure 3B). Notably, circRNA + pA showed higher EGFP expression than IRES-dependent linRNAs or circRNA ΔpA (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, m1ψ; 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 m1ψ 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 (m1ψ) 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 ΔpA showed similar levels of the expression of these genes compared with Acap-linRNA (m1ψ). Interestingly, circRNA + pA decreased the expression of these genes compared with circRNA ΔpA, 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 (m1ψ) maintained normal cell viability, confirming previous observations (42–44). Our circRNAs (circRNA + pA and circRNA ΔpA, 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

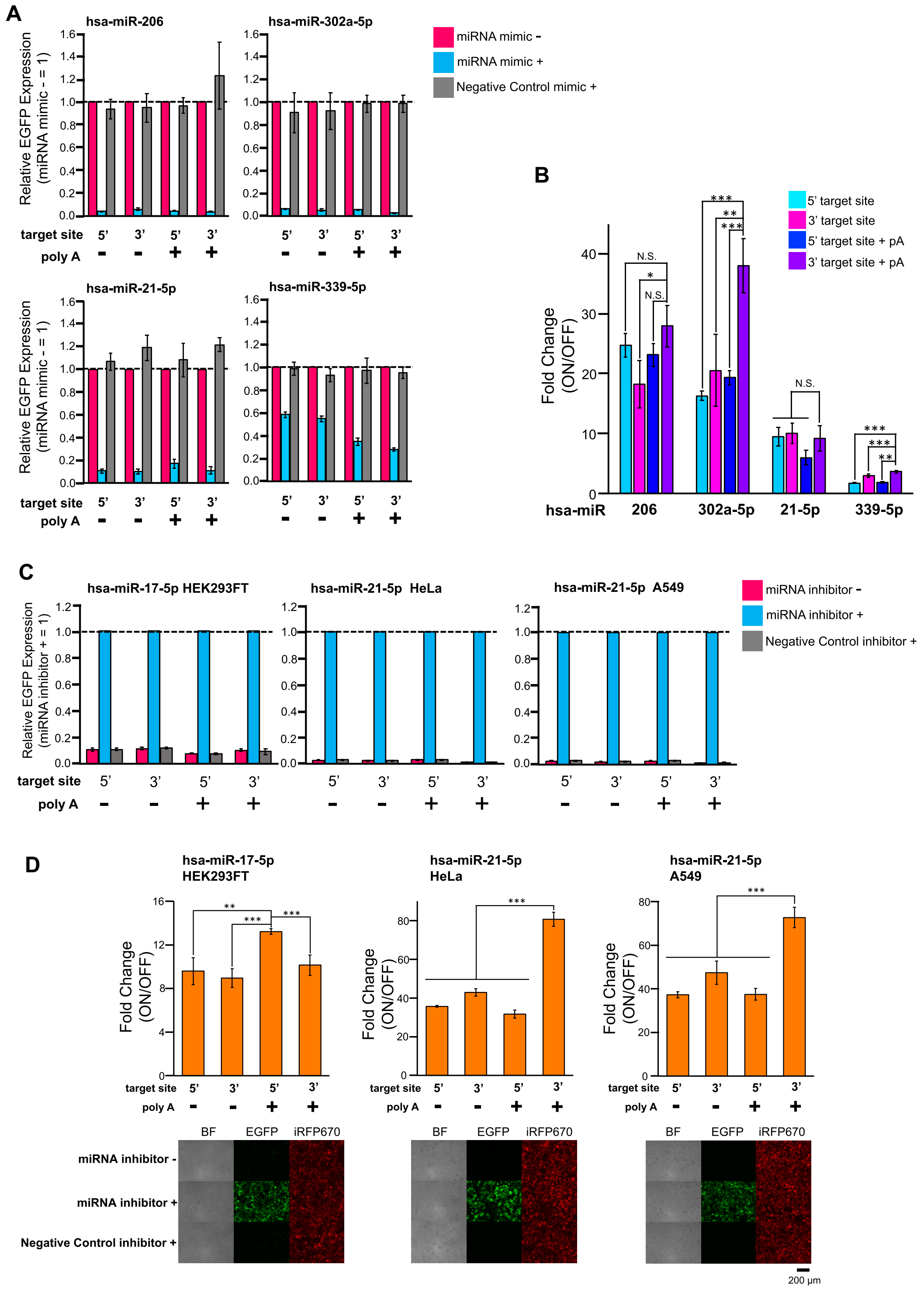

Figure 4. Design and evaluation of miRNA-responsive circRNA switches. (**A**) Evaluation by co-transfecting miRNA mimics in HEK293FT cells. Relative EGFP Expression was calculated by normalizing the sample without a miRNA mimic (magenta). 0.3 pmol of each reporter mRNA and transfection control mRNA were 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 μm. 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 ± SD, $n = 3$.

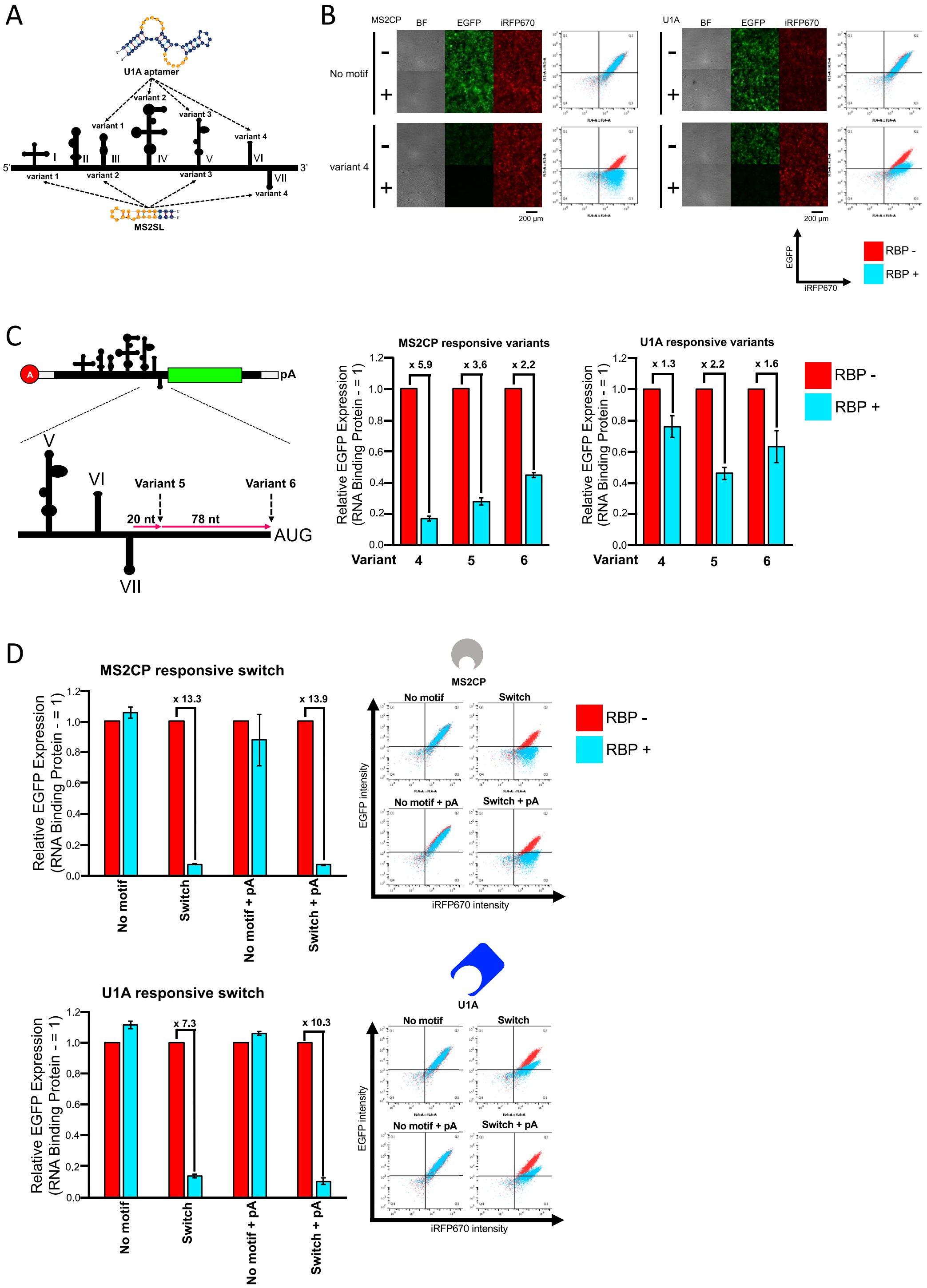

Figure 5. Design and evaluation of protein-responsive circRNA switches. (**A**) Investigation of effective position for the protein-binding motif (aptamer) insertion. Four different variants (variants 1–4) were designed by inserting MS2SL or U1A aptamer. Orange bases in MS2SL and U1A aptamer indicate 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 μm. 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 ± 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 indicates 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 indicates 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 knockdown 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 Metridia 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

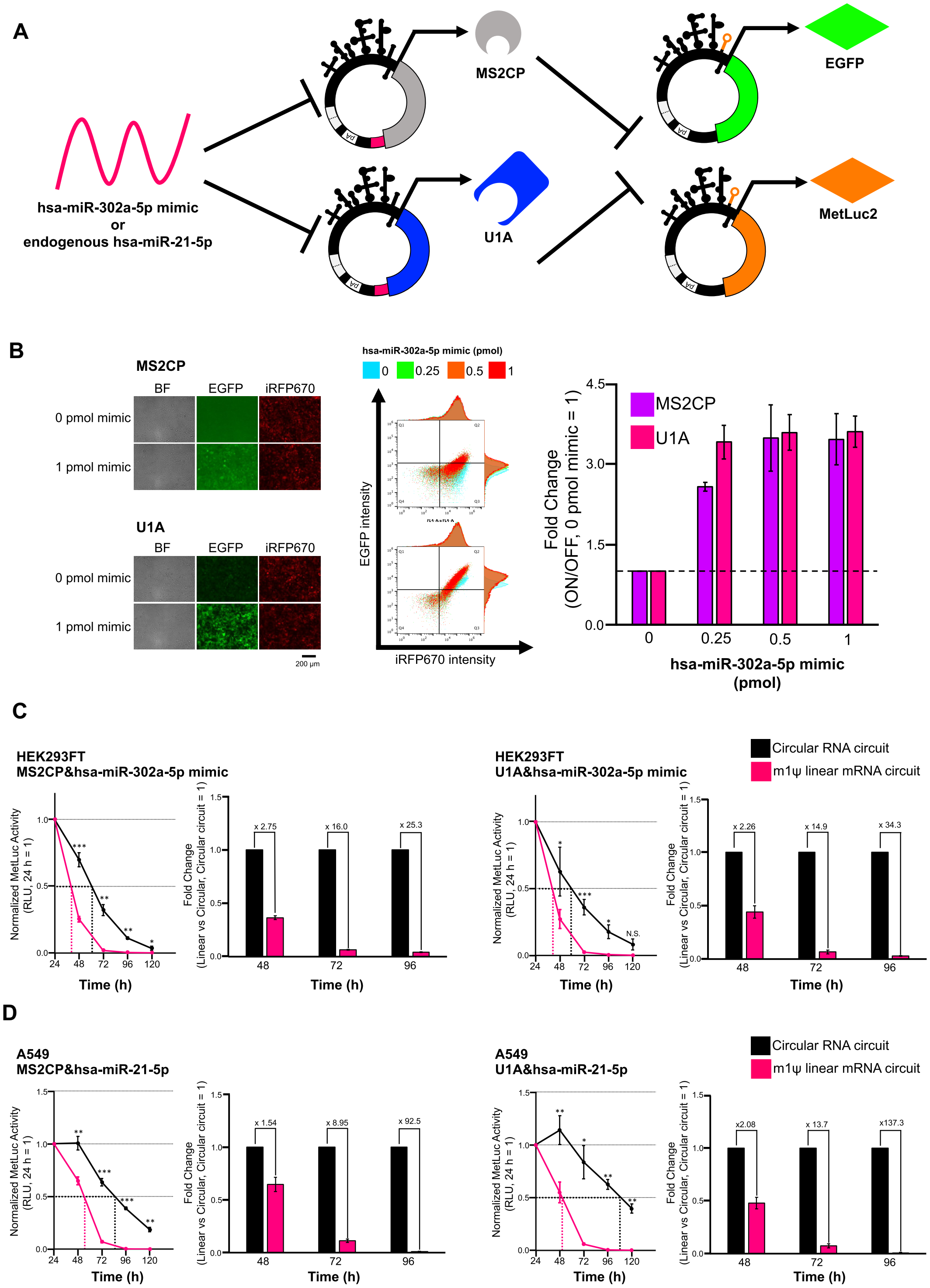

Figure 6. circRNA-based gene circuits that activate translation by detecting miRNA. (**A**) Schematic illustration of miRNA-responsive ON circuit. MS2CP or U1A is encoded on the first miRNA-responsive circRNA switch. The reporter gene (*EGFP* or *MetLuc2*) is encoded on the second protein-responsive 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 μm. 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 ± SD, $n = 3$.

miR-21-5p, respectively. In both cases, the circRNA circuit showed better performance of duration than the linear RNA circuit with m1ψ (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 (m1ψ) 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 m1ψ-substituted linear mRNA switches (Supplementary Figure S11B). In the linear switches, it has been shown that incorporation of m1ψ 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 m1ψ) 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 × 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.* suggested 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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Conflict of interest statement. H.S. is an outside director of aceRNA Technologies, Co., Ltd.

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

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Supplementary Tables (S1, S2)
Supplementary Figures (S1-S13)
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 Ciruclar 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), Cirular 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), Cirular 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 Cirular 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 Cirular 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 Cirular EGFP or MS2CP responsive Circular EGFP variant1～4, 0.05 pmol Cap-MS2CP (m1Ψ) U1A : 0.3 pmol Cirular 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 Cirular EGFP (DpA or pA+) or MS2CP responsive Circular EGFP variant 4 (DpA or pA+), 0.05 pmol Cap-MS2CP (m1Ψ) U1A : 0.3 pmol Cirular 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 Cirular 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 Cirular 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 Cirular 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 Cirular 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

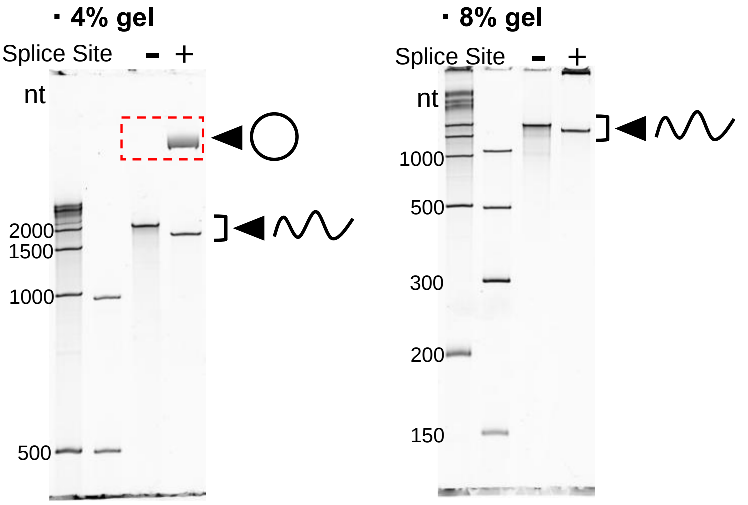


Figure S1. Denaturing polyacrylamide gel images for RNAs after the gel purification. DynaMarker RNA High (BioDynamics Laboratory) and Low Range ssRNA Ladder (NEB) were used as molecular weight markers. circRNA band is shown by red dotted rectangle. While only a minor contamination of nicked circRNA product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

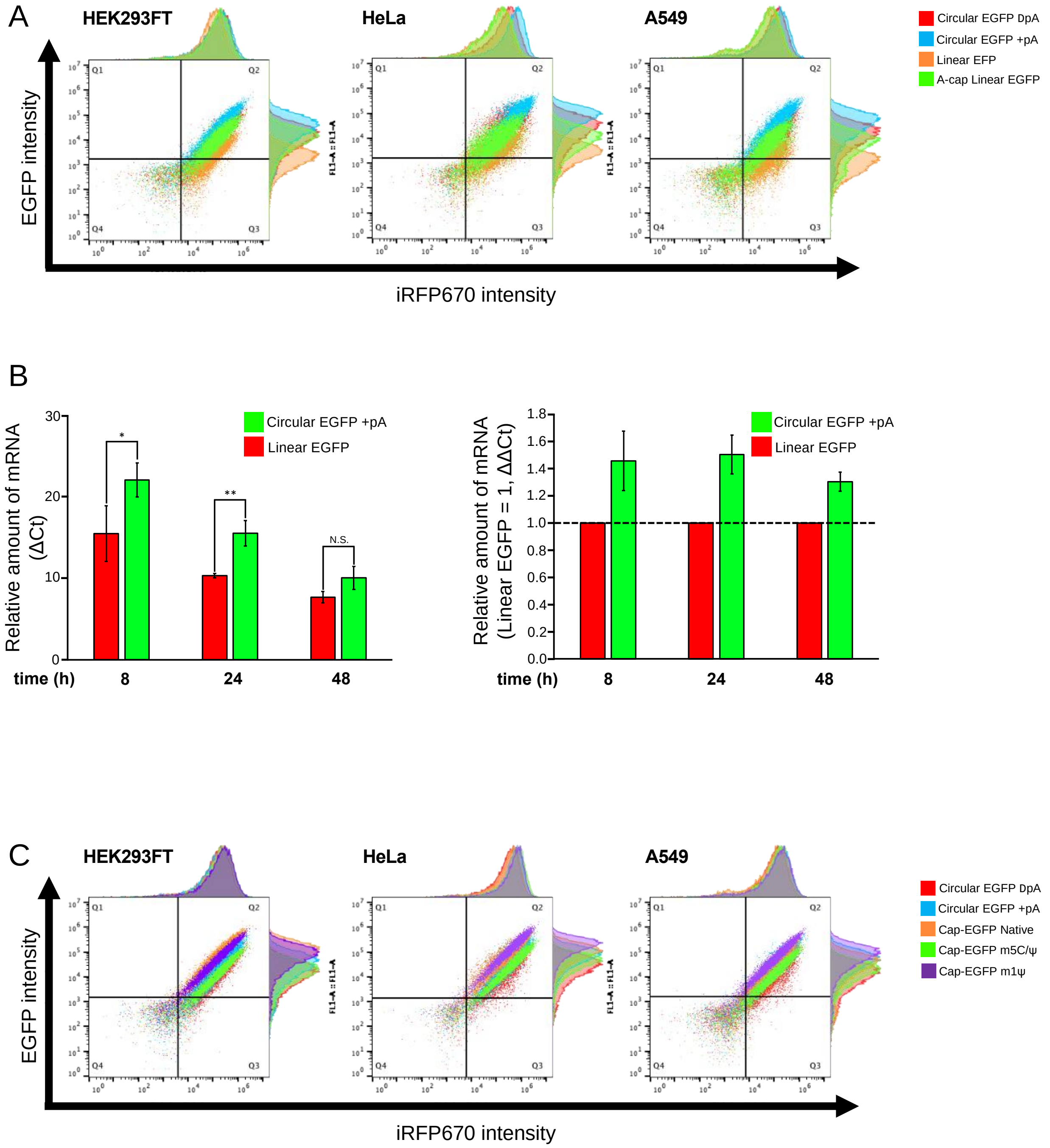
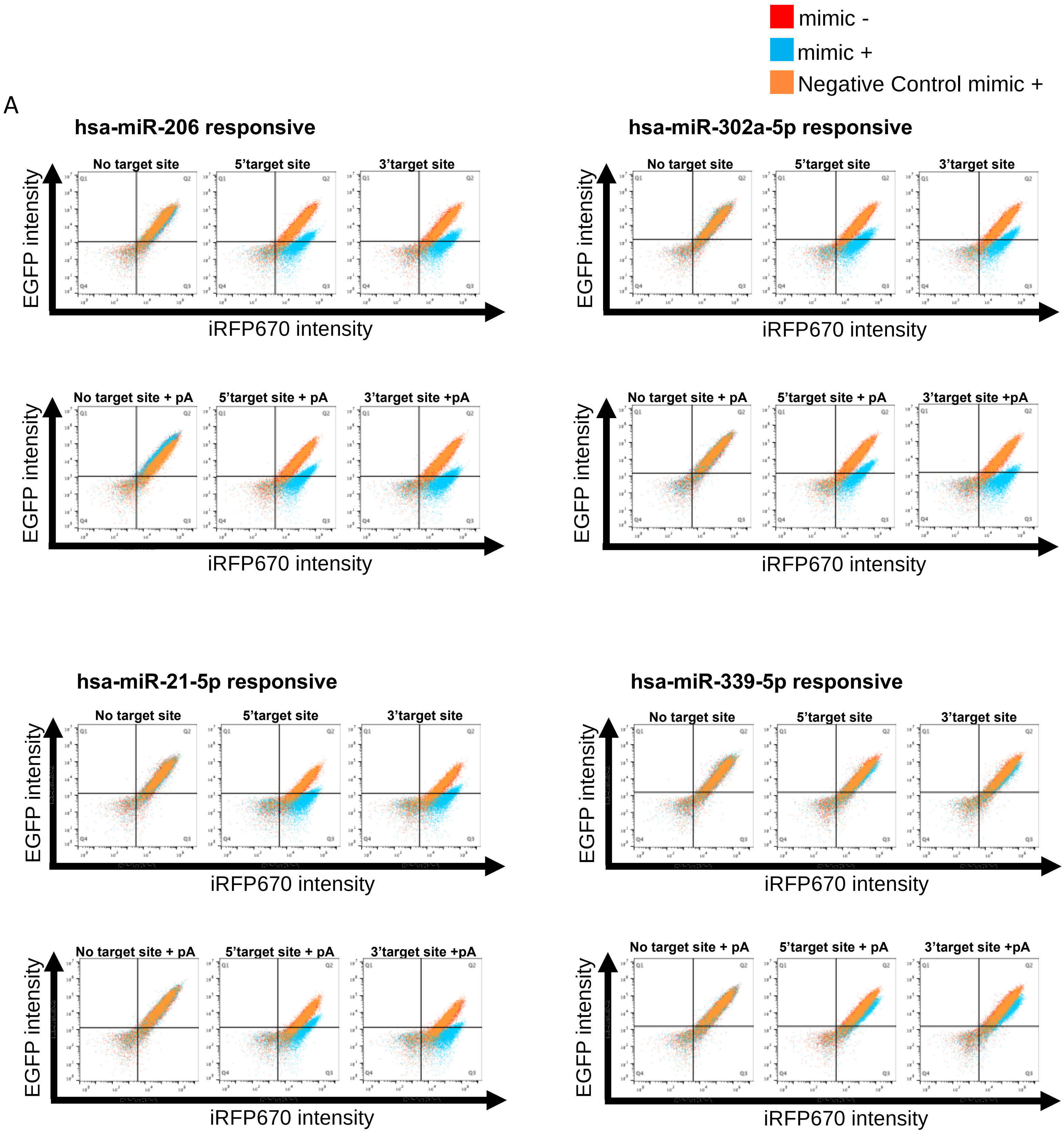
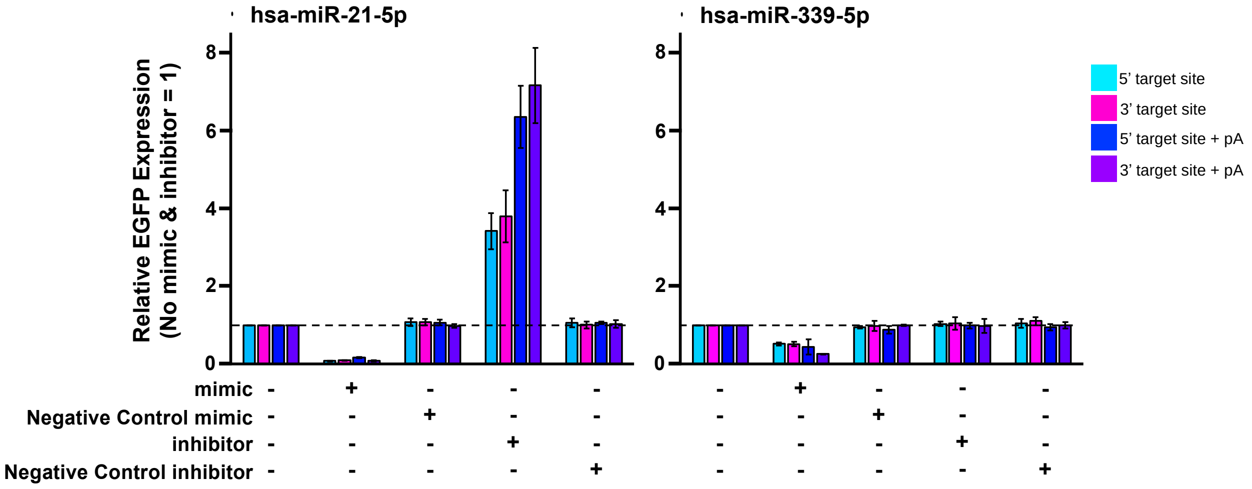


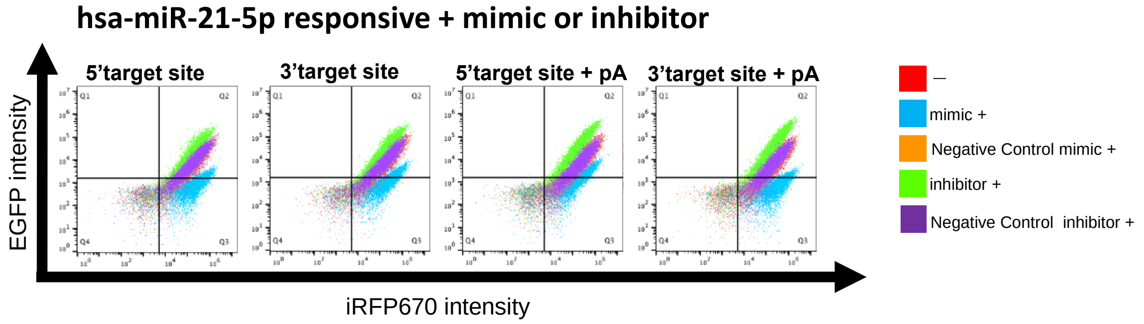
Figure S2. (A) Scatter plots generated from flow cytometry analysis in Figure 3B. (B) Analysis of the relative amount of circRNA and linRNA by RT-qPCR. The residual RNA level was compared between Linear EGFP and Circular EGFP +pA transfection after 8, 24 and 48 h in HEK293FT cells. Target mRNA quantities 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).

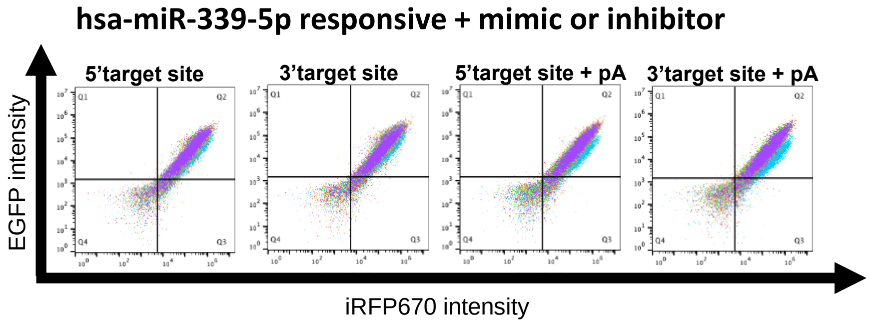
(C) Scatter plots generated from flow cytometry analysis in Figure 3C. All data in this figure are presented as mean ± SD, n = 3. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal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


🟥 mimic -
🟦 mimic +
🟧 Negative Control mimic +
A
hsa-miR-206 responsive
hsa-miR-302a-5p responsive
hsa-miR-21-5p responsive
hsa-miR-339-5p responsive
No target site
5’target site
3’target site
No target site + pA
5’target site + pA
3’target site +pA
EGFP intensity
iRFP670 intensity
Q1
Q2
Q3
Q4

B


hsa-miR-21-5p
hsa-miR-339-5p
Relative EGFP Expression
(No mimic & inhibitor = 1)
0
2
4
6
8
🟦 5' target site
🟪 3' target site
🟦 5' target site + pA
🟪 3' target site + pA
mimic - + - - - - + - - -
Negative Control mimic - - + - - - - + - -
inhibitor - - - + - - - - + -
Negative Control inhibitor - - - - + - - - - +


hsa-miR-21-5p responsive + mimic or inhibitor
5'target site
3'target site
5'target site + pA
3'target site + pA
EGFP intensity
iRFP670 intensity
Q1
Q2
Q3
Q4
🟥 –
🟦 mimic +
🟧 Negative Control mimic +
🟩 inhibitor +
🟪 Negative Control inhibitor +


hsa-miR-339-5p responsive + mimic or inhibitor
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5'target site + pA
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EGFP intensity
iRFP670 intensity
Q1
Q2
Q3
Q4

C

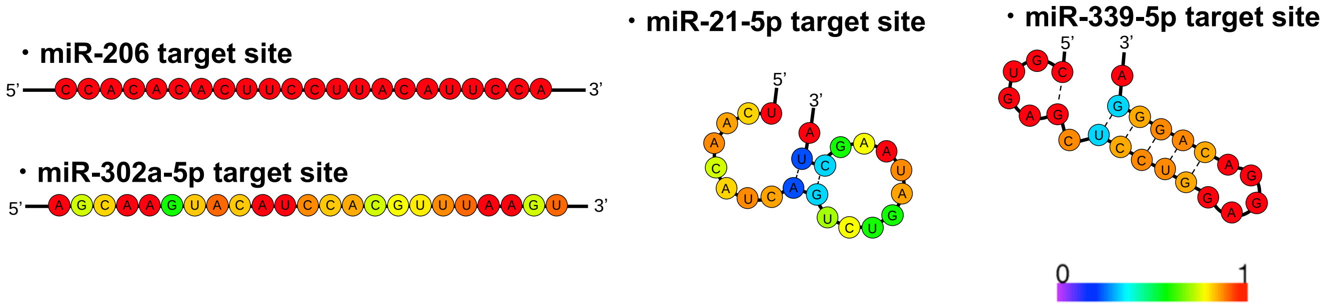

miR-206 target site
5' CCACACACACUUCCUUACAUUCCA 3'
miR-302a-5p target site
5' AGCAAGUACAUCCACGUUUAAGU 3'
miR-21-5p target site
5'
3'
miR-339-5p target site
5'
3'
0
1

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 ± 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.

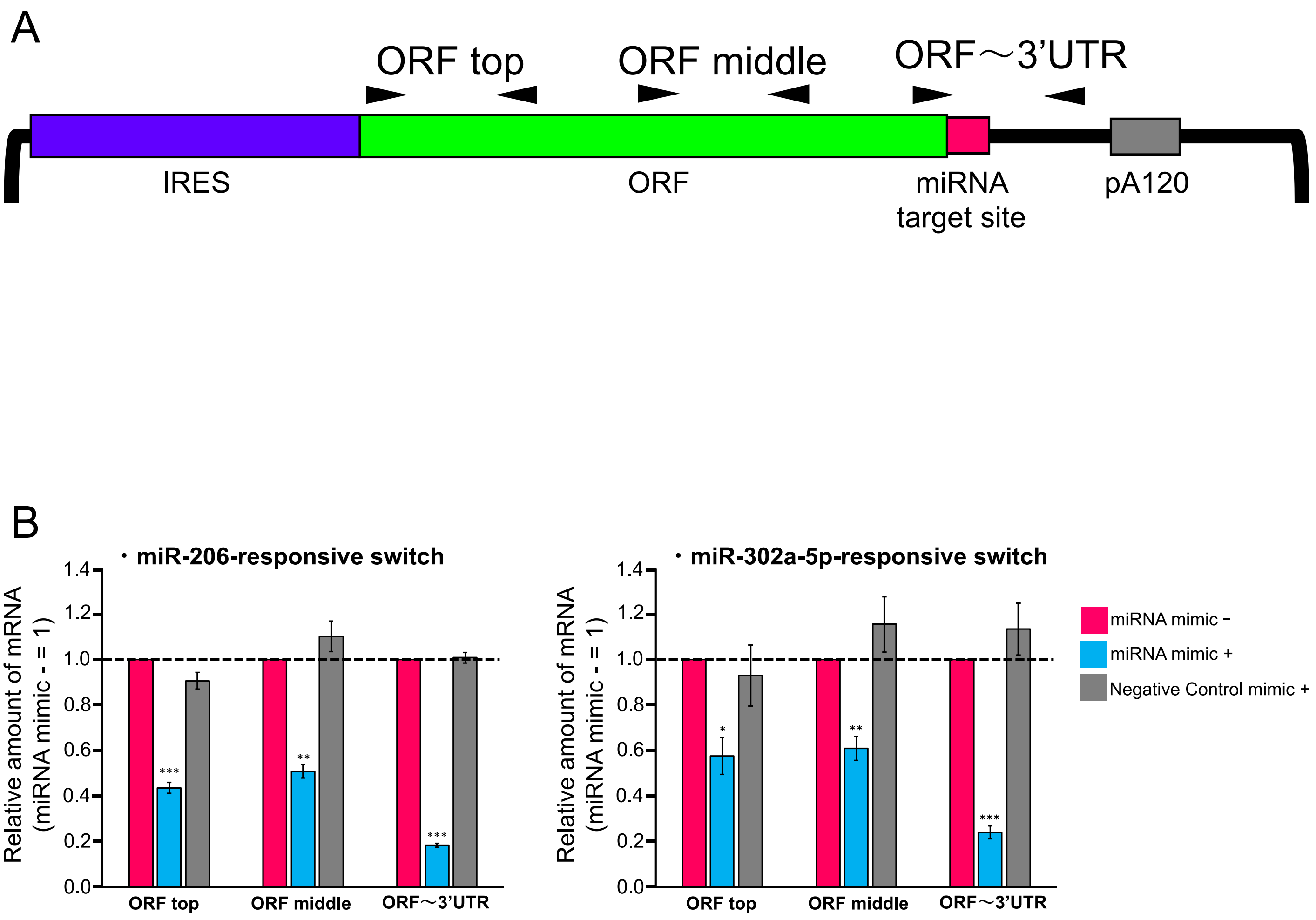


Figure S4. (A) Schematic illustrations of primer pairs on miRNA-responsive circRNA switches. Three different regions were amplified by each primer pair. (B) Evaluation of mRNA amount by RT-qPCR after 24 h of transfection in HEK293FT cells. The relative mRNA amount was calculated by normalizing the sample 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 ± SD, n = 3.

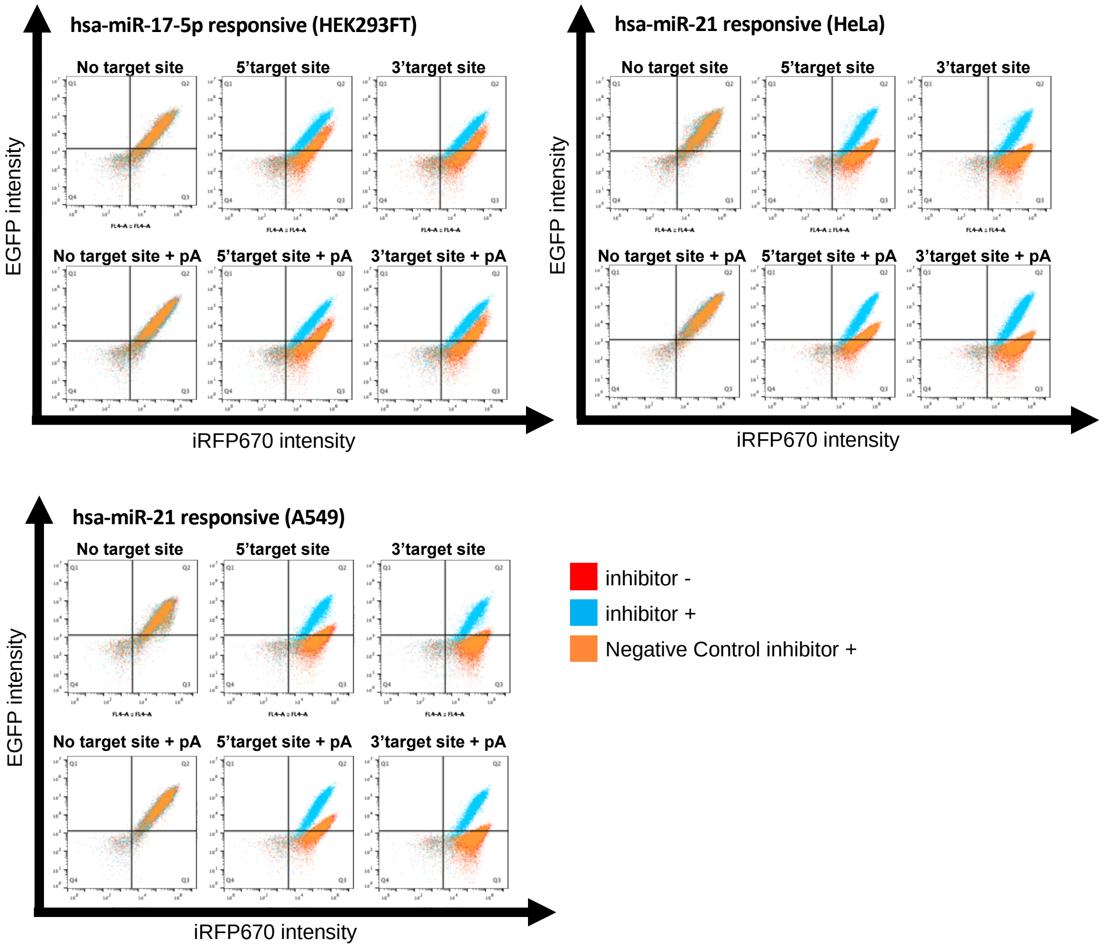


Figure S5. Scatter plots generated from flow cytometry analysis in Figure 4C, 4D. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670.

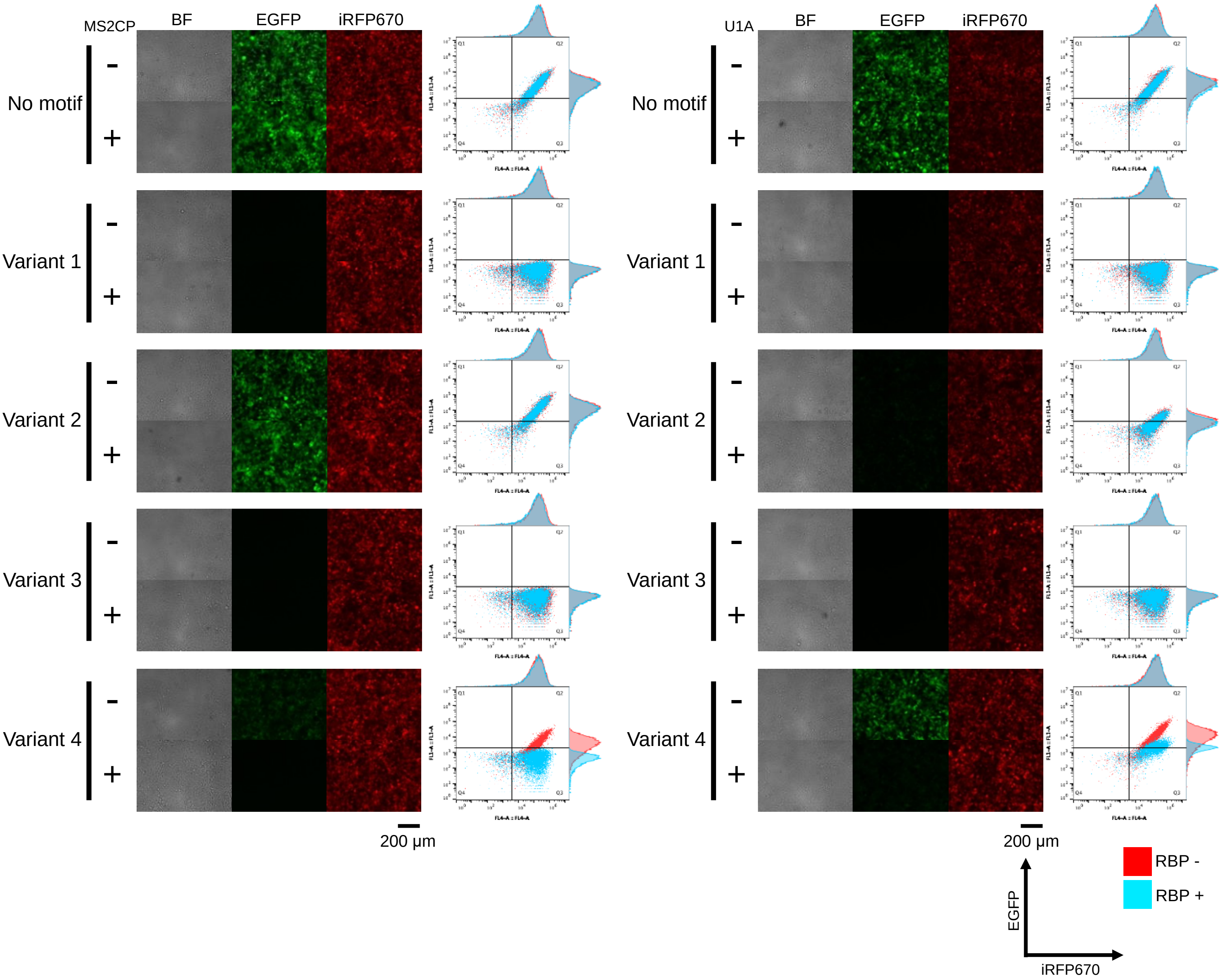


Figure S6. Fluorescent microscopy images and scatter plots from flow cytometry analysis in Figure 5B (including variants 1-4). The scale bar in fluorescent images indicates 200 µm. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the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

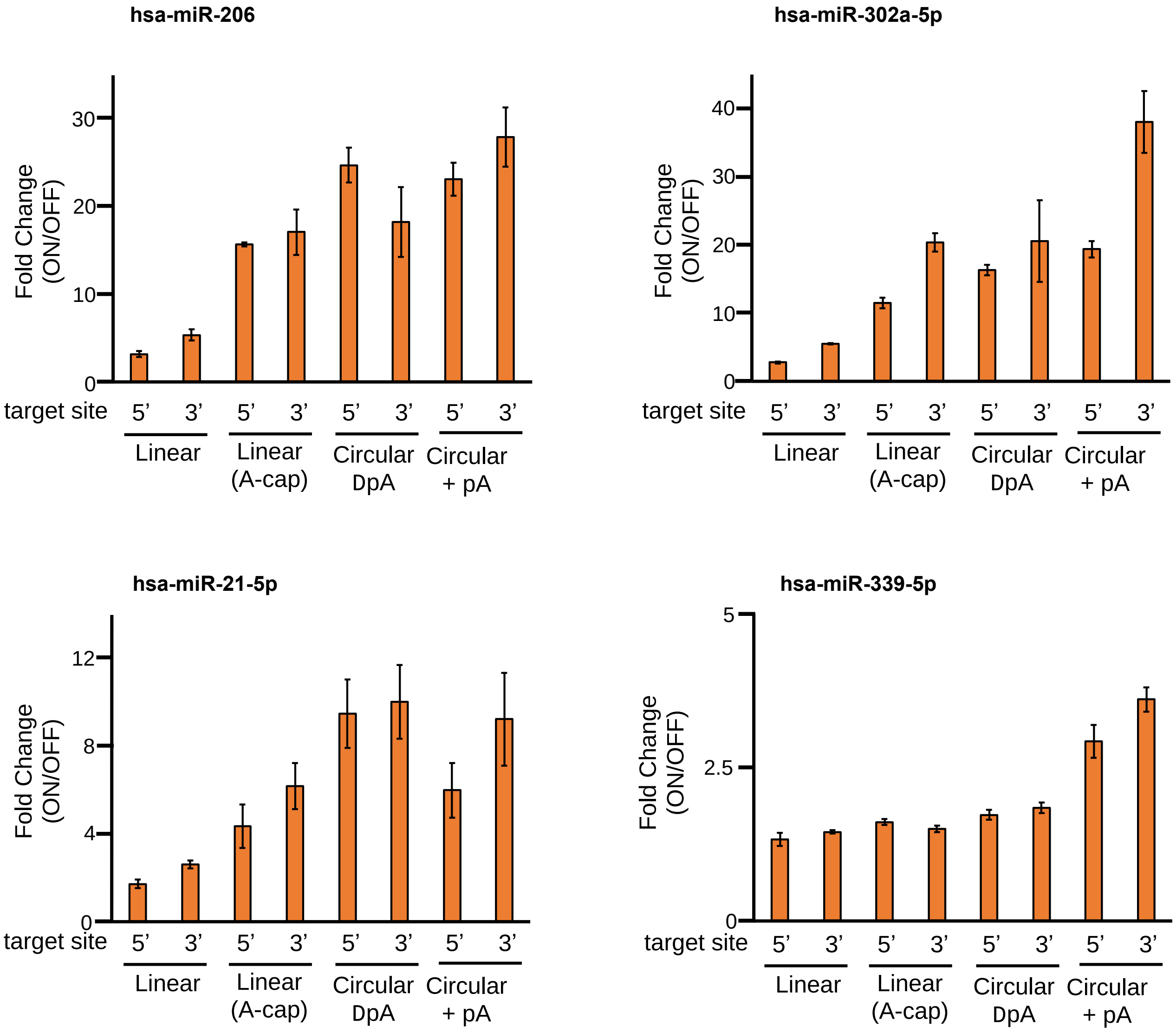
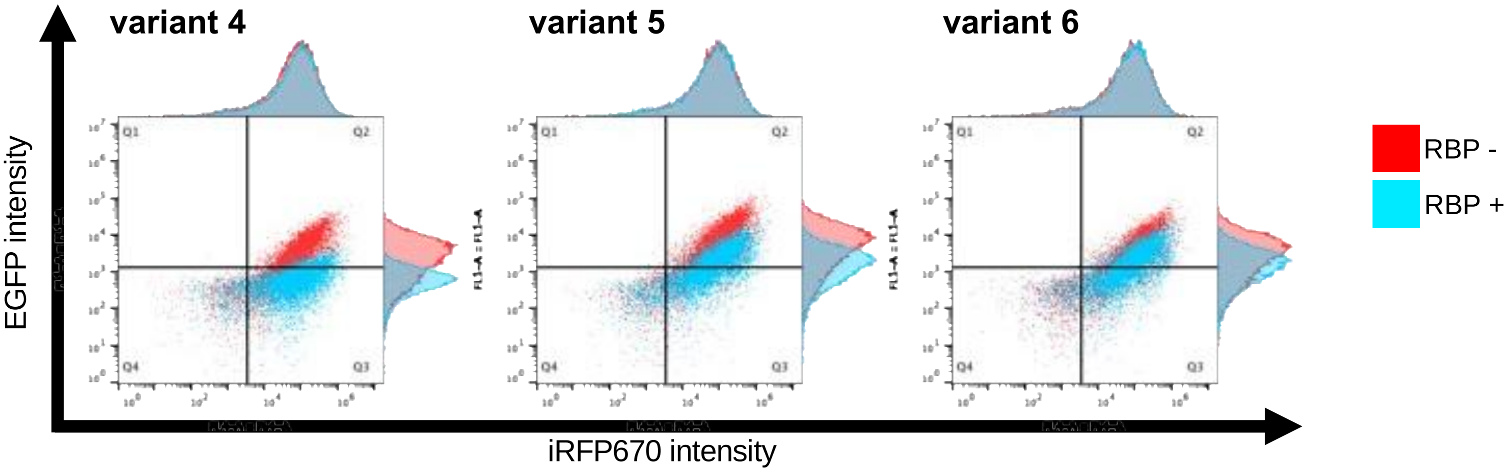


Figure S7. Comparison of miRNA-responsive circRNAs and linear mRNAs with the same sequence components of circRNAs in HEK293FT cells. For circRNA constructs, the results from a single comparison performed simultaneously were presented in two separate graphs, Figure 4B and S7. All data in this figure are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

MS2CP responsive variants



U1A responsive variants

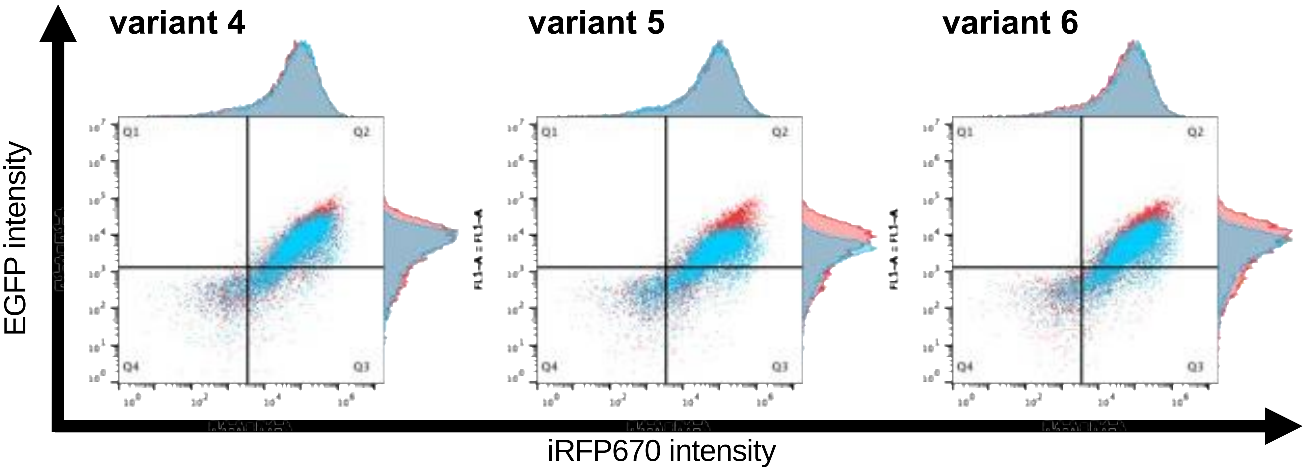


Figure S8. Scatter plots generated from flow cytometry analysis in Figure 5C. The plots shown are representative data from three biological replicates. The vertical axis of the scatter plot shows the fluorescence intensity of EGFP, and the horizontal axis shows the fluorescence intensity of iRFP670.

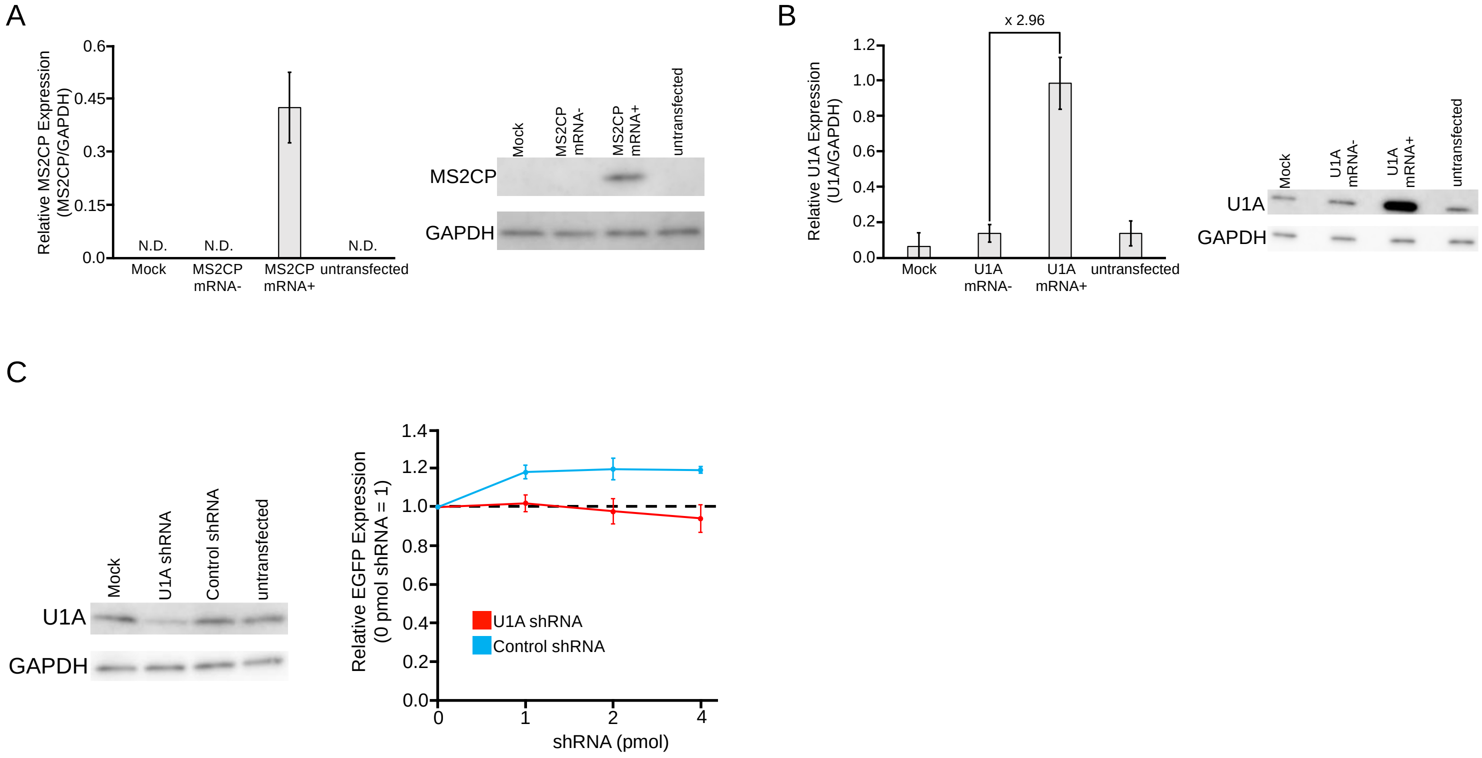


Figure S9. (A) Western blot analysis of MS2CP. 0.05 pmol of *MS2CP*-coding mRNA, 0.3 pmol of each reporter mRNA and transfection control mRNA were co-transfected. Cell culture was performed in 24-well format. (B) Western blot analysis of U1A. 0.15 pmol of *U1A*-coding mRNA, 0.3 pmol of each reporter 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 ± 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).

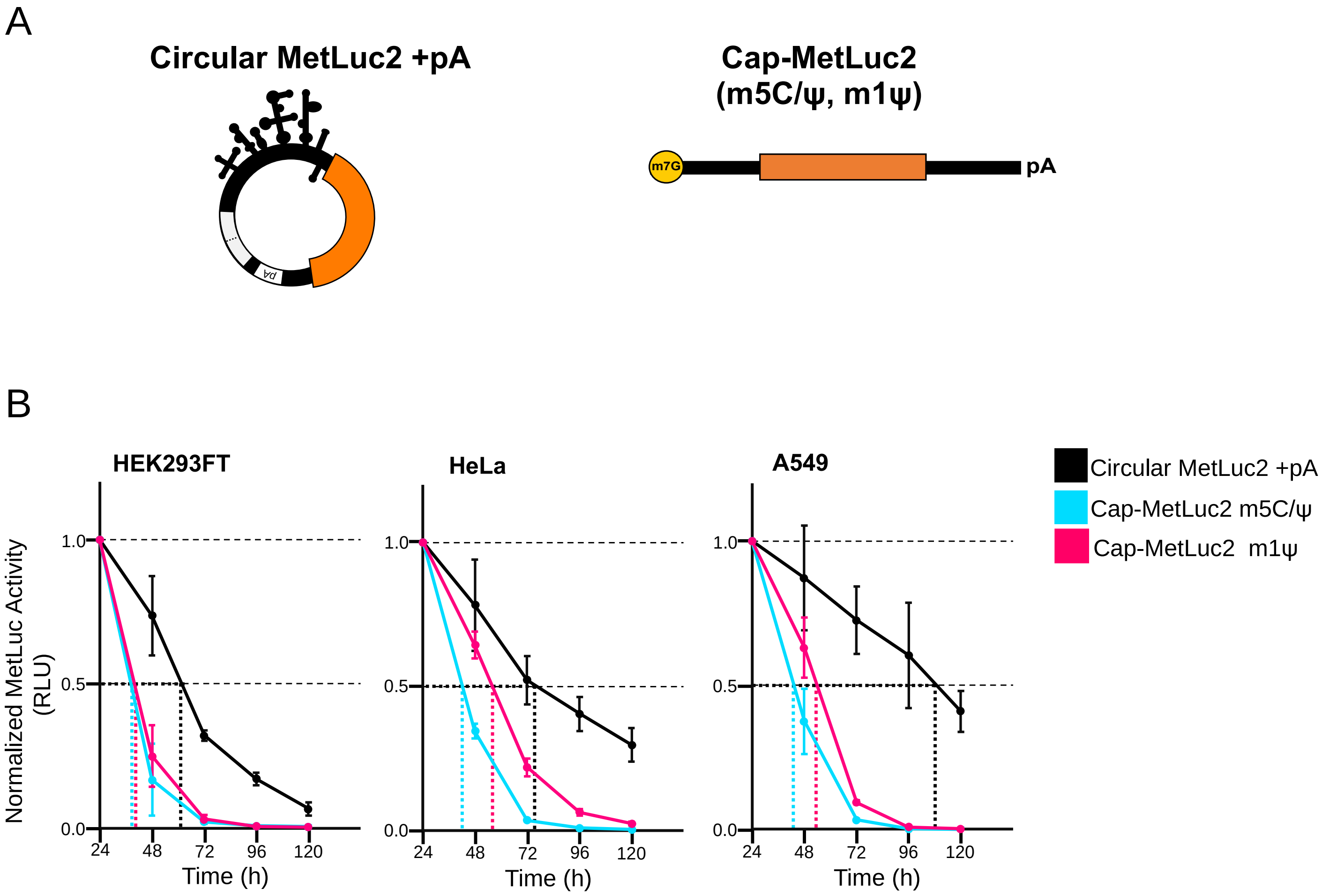


Figure S10. (A) Structure illustration of mRNAs used in the secreted luciferase assay. All mRNAs code Metridia Luciferase (*MetLuc2*) as a reporter gene. (B) Comparison of circRNAs and conventional linear mRNA with base substitutions (m5C/ψ, m1ψ). 45 fmol of reporter mRNA were transfected, and cell culture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

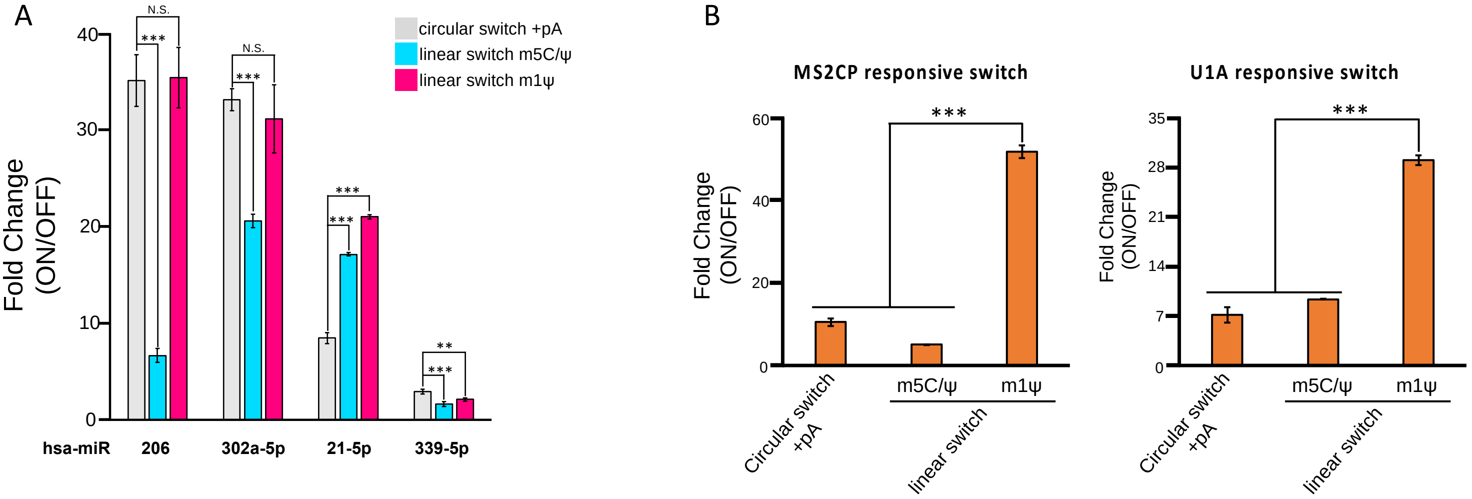


Figure S11. (A) Comparison of the designed miRNA-responsive circRNAs and linear mRNAs with base substitution (m5C/ψ, m1ψ) in HEK293FT cells. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected, and cell culture was performed in 24-well format. 0.25 pmol of specific miRNA mimic 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 ± SD, n = 3.

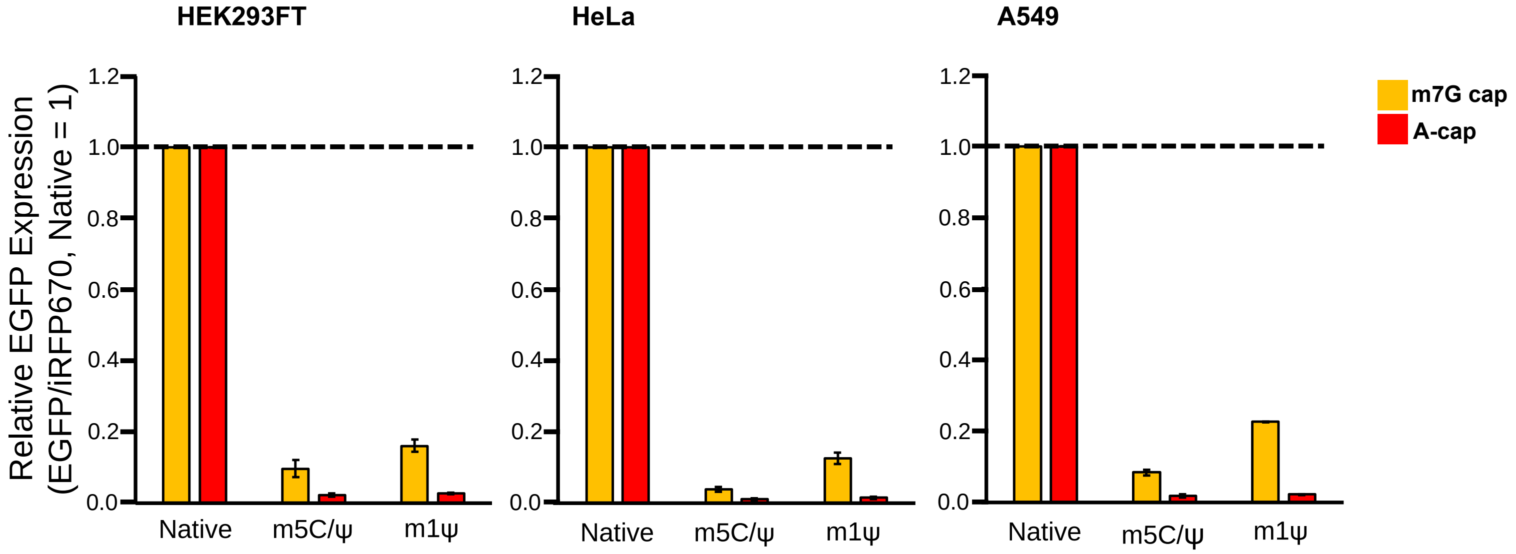


Figure S12. Translatability of linear mRNAs (m7Gcap-EGFP and A-cap-EGFP) with IRES transcribed with non-modified bases (Native) or modified bases (m5C/ψ, m1ψ). 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected, and cell culture was performed in 24-well format. All data in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

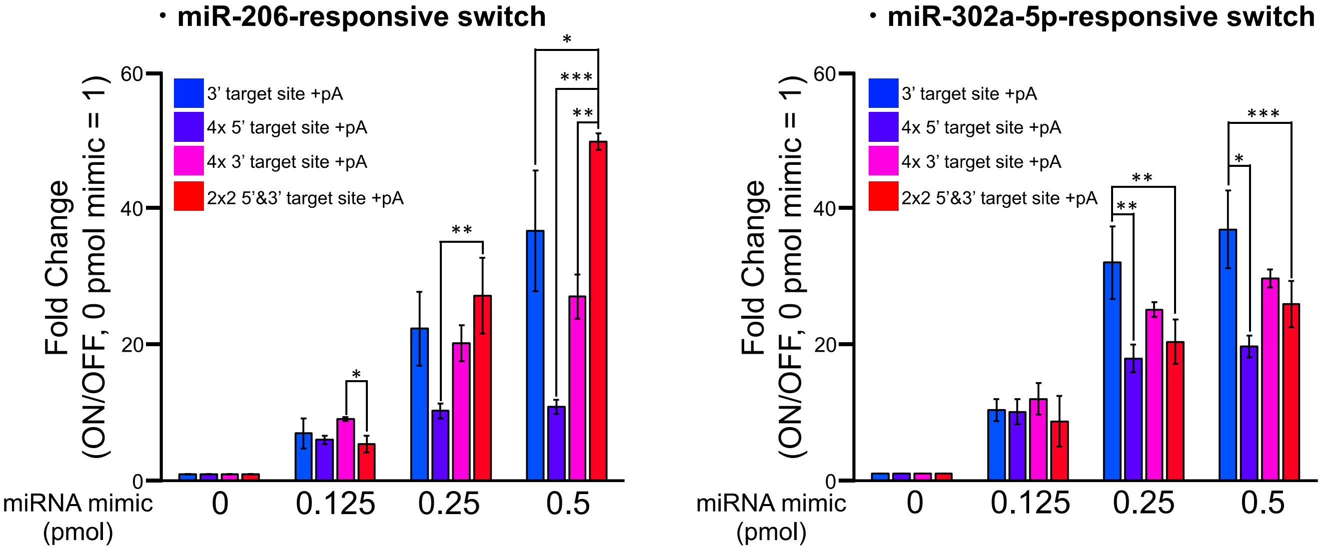


Figure S13. Evaluation of miR-206 or miR-302a-5p-responsive circRNA switches with multiple copies and its patterns of the miRNA target site. 0.3 pmol of each reporter mRNA and transfection control mRNA were transfected and cell culture was performed in 24-well format. 0.125, 0.25, 0.5 pmol of specific 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 ± SD, n = 3.

Supplementary Sequences

Supplementary Sequences. mRNA sequences used in this report

Circular EGFP ΔpA (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP
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Circular EGFP +pA (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP
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Circular EGFP ΔpAΔIRES (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP
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Circular EGFP +pAΔIRES (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP
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Cap-EGFP

EGFP

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Cap-iRFP670

iRFP670

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

Group I intron, CVB3 IRES, MetLuc2

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ugcaccaccggcugccugaagggccuggccaacgugaagugcagcgagcugcugaagaaguggcugcccgacc
gcugcgccagcuucgccgacaagauccagaaagaggugcacaacaucaagggcauggccggcgacaggugauc
uagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuu
GAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUU**
AAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAAC
CUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGAC
CAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAU
UACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCG
AAUU

Cap-MetLuc2
MetLuc2
GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUcgccaccauggac
aucaagguggguguucacccugguguucagcgcccuggugcaggccaagagcaccgaguucgaccccaacaucgacauc
gugggccuggaaggcaaguucggcaucaccaaccuggaaaccgaccuguucaccaucugggagaccauggaagugaug
aucaaggccgacaucgccgacaccgaccgggccagcaacuucguggccaccgagaccgacgccaaccggggcaagaugc
ccggcaagaagcugccccuggccgucaucauggaaauggaagccaacgccuucaaggccggcugcacccggggcugccu
gaucugccugagcaagaucaagugcaccgccaagaugaagguguacauccccggcaggugccacgacuacggcggcga
caagaaaaccggccaggccggcaucgugggcgccaucguggacauccccgagaucagcggcuucaaagaaauggccccc
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ugcagcgagcugcugaagaaguggcugcccgaccgcugcgccagcuucgccgacaagauccagaaagaggugcacaac
aucaagggcauggccggcgacaggugaucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuu
gcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCCCACAC
ACUUCCUUACAUUCCAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCC
CAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCC
UCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCA
GCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCAC
GCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAAC
ACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAU
GAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCA
UGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUA
GUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCU
CAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUG
GGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU
CGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCC
CUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUU
AAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauc
cuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccacc
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agaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcaug
gacgagcuguacaagagaucucaugcaucucgagugauagucuagaccuucugcggggcuugccuucugg
ccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCU
AUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAU
UCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAU

CCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCCCACACACUUCCUUACAUUCCAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
GGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAG
AAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGG
CAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAAC
AGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGC
AGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3’T206 Circular EGFP ∆pA (**bold: circularized**)

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
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UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
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cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
ugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
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acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
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gccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGC
UAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAA
UUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAA
UCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGC
AUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
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UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
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ugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug

cugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacau
gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagCCACACACUUCCUUACAUUCCAucuagaccuucugcggggcuugccuucug
gccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAA
GAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAG
GCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAA
CAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUG
CAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCCCACAC
ACUUCCUUACAUUCCACCACACACUUCCUUACAUUCCACCACACACUUCCUUACAUUC
CACCACACACUUCCUUACAUUCCAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACC
CACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUU
AUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUG
GCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGA
CUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAAC
CUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUC
AGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGG
CCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUA
UUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCA
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CUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAU
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ggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggccca

cccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuu
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acccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaagg
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gcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgac
cacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguc
cgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggauc
acucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcgggg
cuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGA
GUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCC
UUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUA
UAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCG
ACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUG
CAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

4x 3’T206 Circular EGFP +pA (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP, miR-206 target site
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU

UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagCCACACACUUCCUUACAUUCCACCACACACUUCCUUACAUUCCACCACACACUUCCUUACAUUCCACCACACACUUCCUUACAUUCCAucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCCCACACACUUCCUUACAUUCCACCACACACUUCCUUACAUUCCAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGG**

UGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAU
GGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAU
CCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACU
CUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCU
GCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGU
GACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAG
GUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggc
gaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagc
guguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagc
ugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgacca
caugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaag
gacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcuga
agggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgu
cuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggc
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acuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacauggucnugcuggaguu
cgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugaua
gCCACACACUUCCUUACAUUCCACCACACACUUCCUUACAUUCCAucuagaccuucugcggg
gcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUG
AGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGC
CUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUU
AUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUC
GACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUU
GCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCAGCAAG
UACAUCCACGUUUAAGUGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGC

CCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCC
CUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACC
AGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCA
CGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAA
CACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGA
UGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCC
AUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCU
AGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCC
UCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
GGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGA
UCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAUC
CCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGU
UAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugccca
uccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugcca
ccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugac
cacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaagucc
gccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccg
aggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggca
acauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaa
cggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccag
cagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccuga
gcaaagaccccaacgagaagcgcgaucacauggucgugcuggaguucgugaccgccgccgggaucacucucgg
cauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuu
cuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGG
GCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGA
AAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGC
AAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACA
GCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCAGCAAG

UACAUCCACGUUUAAGUGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGC
CCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCC
CUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACC
AGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCA
CGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAA
CACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGA
UGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCC
AUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCU
AGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCC
UCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
GGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGA
UCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUC
CCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGU
UAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugccca
uccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugcca
ccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugac
cacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaagucc
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acauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaa
cggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccag
cagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccuga
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cauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuu
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AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAG
AAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACA
AGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAU
AACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCC
UGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3’T302*a*-5p Circular EGFP ∆pA (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP, miR-302a-5p target site
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG

GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
ugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
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caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacau
gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagAGCAAGUACAUCCACGUUUAAGUucuagaccuucugcggggcuugccuuc
uggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGG
CUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAA
AUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCA
AUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAG
CAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
ugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgcca
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gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagAGCAAGUACAUCCACGUUUAAGUucuagaccuucugcggggcuugccuuc
uggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGA
AGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAA
GGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUA
ACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCU

GCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCAGCAAG
UACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACGUU
UAAGUAGCAAGUACAUCCACGUUUAAGUGCCACCUUAAAACAGCCUGUGGGUUGAUC
CCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCU
GUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCA
GCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUC
AAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCG
AAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUG
UAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGU
UGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAG
AGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGC
GGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGG
AACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGU
GACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAG
AGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACA
UUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguu
caccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcga
gggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugccc
uggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagc
acgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaa
cuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgac
uucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucaugg
ccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcu
cgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagca
cccaguccgcccugagcaaagaccccaacgagaagcgcgaucacauggucCugcuggaguucgugaccgccgc
cgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuuc
ugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAA
GCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAU
UGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUC
UAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGA
CAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCA
AGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
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UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
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cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
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gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacau

gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAG
UAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUucuagaccuucug
cggggcuugccuucuggccaugcccuucuucucccuugcaccuguaccucuuggucuuuGAAUAAAGC
CUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUG
AGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUA
GUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACA
AUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAG
CUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCAGCAAG
UACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUGCCACCUUAAAACAGCCU
GUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACC
UUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCG
AUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCG
GACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGC
CAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCA
CUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGC
GGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGAC
AUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUA
AUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAA
CUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGG
CUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCG
GUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAG
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ggcgaggagcuguucaccggggugugcccauccuggucgagcuggacggcgacguaaacggccacaaguuc
agcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggca
agcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccga

ccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuuc
aaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagc
ugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaa
cgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggac
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accacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcugga
guucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagug
auagAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUucuagaccuuc
ugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAA
GCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAU
UGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUC
UAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGA
CAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCA
AGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

5'T21-5p Circular EGFP ΔpA (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP, miR-21-5p target site
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCUCAACA
UCAGUCUGAUAAGCUAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGC
CCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCC
CUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACC
AGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCA
CGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAA
CACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGA
UGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCC
AUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCU
AGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCC
UCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
GGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGA
UCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUC

CCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCUCAACAUCAGUCUGAUAAGCUAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGA**

**UCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUC
CCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGU
UAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugccca
uccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugcca
ccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugac
cacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaagucc
gccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccg
aggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggca
acauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaa
cggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccag
cagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccuga
gcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucgg
cauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuu
cuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUU**AAAUAAUUGAGCCUUAAAG
AAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACA
AGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAU
AACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCC
UGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3’T21-5p Circular EGFP ΔpA (**bold: circularized**)

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU**

CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
ugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacu
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gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagUCAACAUCAGUCUGAUAAGCUAucuagaccuucugcggggcuugccuucu
ggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGG
CUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAA
AUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCA
AUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAG
CAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG

GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
ugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacau
gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagUCAACAUCAGUCUGAUAAGCUAucuagaccuucugcggggcuugccuucu
ggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAA
GAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAG
GCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAA
CAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUG
CAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

5’T339-5p Circular EGFP ∆pA (**bold: circularized**)

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCcgugagcu
ccuggaggacagggaGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUU
GGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCC

CCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCA
CGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCG
GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACC
GUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAG
UCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGG
GGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUU
GGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAA
GCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGU
GUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGU
UACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUU
UGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAG
UUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccug
gucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuac
ggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccaccc
ugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccau
gcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggug
aaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacaucc
uggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcau
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accccaacgagaagcgcgaucacauggucugcuggaguucgugaccgccgccgggaucacucucggcaugga
cgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggcc
augcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAU
UAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUC
UUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCC
UGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUA
UCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCcgugagcu
ccuggaggacagggaGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUU

GGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCUCCC
CCAACUGUAACUUAGAAGUAACACACCGAUCAACAGUCAGCGUGGCACACCAGCCA
CGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCG
GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACC
GUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAG
UCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGG
GGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUU
GGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAA
GCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGU
GUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGU
UACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUU
UGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAG
UUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccug
gucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuac
ggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccaccc
ugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccau
gcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggug
aaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacaucc
uggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcau
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acccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaag
accccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcaugga
cgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggcc
augcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGG
CUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAA
AUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCA
AUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAG
CAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAG
GUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

3’T339-5p Circular EGFP ∆pA (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP, miR-339-5p target site
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG

UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
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aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacu
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gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagcgugagcuccuggaggacagggaucuagaccuucugcggggcuugccuucuggcca
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UAUGCGUUACCGGCGAGACGCUACGGACUU**AAAUAAUUGAGCCUUAAAGAAGAAAUUC
UUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCC
UGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUA
UCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG

GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
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gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
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acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacau
gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagcgugagcuccuggaggacagggaucuagaccuucugcggggcuugccuucuggcca
ugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGC
UAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAA
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UCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGC
AUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGG
UCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCcuaccugc
acuguaagcacuuugGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUU
GGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCC
CCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCA
CGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCG
GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACC
GUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAG
UCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGG
GGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUU
GGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAA
GCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGU
GUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGU
UACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUU
UGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAG
UUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccug
gucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuac
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gcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggug
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cgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggcc
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UAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUC
UUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCC
UGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUA

UCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCcuaccugc
acuguaagcacuuugGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUU
GGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCC
CCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCA
CGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCG
GUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACC
GUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAG
UCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGG
GGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUU
GGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAA
GCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGU
GUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGU
UACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUU
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UUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccug
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ggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccaccc
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cgagcuguacaagagaucucaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggcc
augcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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CUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAA
AUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCA
AUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAG
CAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAG
GUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg
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cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
ugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
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acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgcca
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cugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacau
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gcaucucgagugauagcuaccugcacuguaagcacuuugucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccugguggaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacau**

gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagcuaccugcacuguaagcacuuugucuagaccuucugcggggcuugccuucuggcca
ugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGC
UAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAA
UUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAA
UCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGC
AUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGG
UCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

5’T206 Linear EGFP

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCCCACACACUUCCUUACAUUCCAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCA
CCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUU
UAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUG
GCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGAC
UGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUA
GUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGU
CGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUG
CCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAG
CUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACC
CUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
GGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU
CGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCC
UUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAA
GUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucg
agcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcuga
cccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugca
gugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggag
cgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaacc
gcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagcc
acaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacg
gcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuac

cugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacauggucc ugcuggaguucgugaccgccg
ccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcgg
ggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUA
GGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

3’T206 Linear EGFP

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAAAAGAAGAGUAAGAAAUAUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUU
AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggaucc
gugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaag
uucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagc
ugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaa
gcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaac
uacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaag
gaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcag
aagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagc
agaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaaga
ccccaacgagaagcgcgaucacauggucc ugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcug
uacaagagaucucauaugcaucucgagugauagCCACACACUUCCUUACAUUCCAucuagaccuucugcgg
ggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUA
GGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAA

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

5’T302a-5p Linear EGFP

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCAGCAAGUACAUCCACGUUUAAGUGCCACCUUAAAACAGCCUGUGGGUUGAUCCC
ACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUU
UUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGU
GGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGA
CUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCU
AGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGG
UCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCU
GCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGA
GCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACAC
CCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUU
UGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGA
UCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCC
CUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUA
AGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccugguc
gagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcug
acccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugc
agugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccagga
gcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaa
ccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacag
ccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgagga
cggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacu
accugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgc
cgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugc
ggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAG
UAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

3’T302a-5p Linear EGFP

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAAUAUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUU
AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggaucc
gugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaag
uucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagc
ugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaa
gcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaac
uacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaag
gaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcag
aagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagc
agaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaaga
ccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcug
uacaagagaucucauaugcaucucgagugauagAGCAAGUACAUCCACGUUUAAGUucuagaccuucugcg
gggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGU
AGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

5’T21-5p Linear EGFP

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAAUAUAAGACAC
CGGUCUCAACAUCAGUCUGAUAAGCUAGCCACCUUAAAACAGCCUGUGGGUUGAUCCCA

CCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUU
UAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUG
GCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGAC
UGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUA
GUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGU
CGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUG
CCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAG
CUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACC
CUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUU
GGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAU
CGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCC
UUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAA
GUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucg
agcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcuga
cccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugca
gugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggag
cgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaacc
gcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagcc
acaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacg
gcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuac
cugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacauggccugcuggaguucgugaccgccg
ccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcgg
ggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUA
GGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

3’T21-5p Linear EGFP

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA

GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUU
AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggaucc
gugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaag
uucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagc
ugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaa
gcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaac
uacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaag
gaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcag
aagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagc
agaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaaga
ccccaacgagaagcgcgaucacauggccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcug
uacaagagaucucauaugcaucucgagugauagUCAACAUCAGUCUGAUAAGCUAucuagaccuucugcgg
ggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUA
GGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

5’T339-5p Linear EGFP

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCcgugagcuccuggaggacagggaGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCA
CAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUA
CCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCAC
ACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCU
CACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAA
CACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAU
GAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCA
UGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAG
UUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCA

AGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGU
GUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUU
ACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUG
UUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUG
AAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcugg
acggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccuga
aguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuu
cagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcacc
aucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcauc
gagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaac
gucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagc
gugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccuga
gcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgg
gaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcu
ugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGG
GGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

3’T339-5p Linear EGFP

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUU
AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggaucc

gugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaag
uucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagc
ugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaa
gcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaac
uacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaag
gaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcag
aagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagc
agaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaaga
ccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcug
uacaagagaucucauaugcaucucgagugauagcgugagcuccuggaggacagggaucuagaccuucugcggggcuug
ccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGG
CUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

linear switch (miR-206)

EGFP, miR-206 target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUcCCACACACU
UCCUUACAUUCCAgccaccaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccugg
ucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagc
ugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgu
gcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccag
gagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaac
agccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgag
gacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaacc
acuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugac
cgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuuc
ugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUG
AGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

linear switch (miR-302a-5p)

EGFP, miR-302a-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUcAGCAAGUAC
AUCCACGUUUAAGUgccaccaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccug

gucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaag
cugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcg
ugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacgucca
ggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggu
gaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaa
cagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucga
ggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaac
cacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucguga
ccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuu
cugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCU
GAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
A

linear switch (miR-21-5p)

EGFP, miR-21-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUcUCAACAUCA
GUCUGAUAAGCUAgccaccaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccugg
ucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagc
ugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgu
gcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccag
gagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaac
agccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgag
gacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaacc
acuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugac
cgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuuc
ugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUG
AGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

linear switch (miR-339-5p)

EGFP, miR-339-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUccgugagcuccug
gaggacagggagccaccaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagc
uggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugaccc

ugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagug
cuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgc
accaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgc
aucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccac
aacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggc
agcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccu
gagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgcc
gggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcgggg
cuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAG
GAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCGGGAGCAGGUGAGGAUCACCCAUCUGCCACGAGCGAGGUGAGGAUCAC
CCAUCUCGCUCGUGUUCCCUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGU
GGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUC
ACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGG
AAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGG
UAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGC
CAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGU
CCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUA
CCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUG
UUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUU
GAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccugguc
gagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggc
aagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccuga
ccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcc

cgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaag
uucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccugg
ggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaa
ggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacc
cccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagacc
ccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacga
gcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaug
cccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUA
UGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUU
UAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUG
AGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUC
UAG

MS2CP responsive Circular EGFP variant2 (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP, MS2CP binding motif
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUGGUGAGGAUCACCCAUCCACCCACAGGCCCGGTGAGGAT
CACCCATCGGGCGCTAGCACTCTGGTAGGUGAGGAUCACCCAUCUACCUUUGUGCGCC
UGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUC
AGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAU
CAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUC
GAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGU
GUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCG
UUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAA
GAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUG
CGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCG
GAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGG
UGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUA
GAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAAC
AUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcugu
ucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcg
agggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcc

cuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCGCAGGUGAGGAUCACCCAUCUGCCACGAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggcc**

acaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcac
caccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgc
uaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcacca
ucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccg
caucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaac
agccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaaca
ucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcu
gcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccc
ugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcauc
ucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccugua
ccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUA
CGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCA
GGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUA
GUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGCAGGUGAGGAUCACCCAUCUGCCAC
GAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUUCGAUCGUUACCAUAUAGCUAUUG
GAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACC
ACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAa

ugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacg
uaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguu
caucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugc
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agcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccu
ggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguac
aacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagaucc
gccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggcccc
gugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgauc
acauggucugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucuc
auaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuu
gcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCG
AGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCU
CAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGU
AGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCGACAGCAUUGUACCCAGAGUCUGUCCCCAGACAUUGCACCUGGCGCUG
UCUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggg

auccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaa
cggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucauc
ugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuuca
gccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcg
caccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggug
aaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacu
acaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgcca
caacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgug
cugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacau
gguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauau
gcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcac
cuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGAC
GCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAA
CUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUA
AUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAUUGUACUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGAUUGCACGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCC
UGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGU
CGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUA
UUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGG
AUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCAC
UUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaug

ggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacgua
aacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguuca
ucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuu
cagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggag
cgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccugg
ugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaa
cuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgc
cacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccg
ugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucac
augguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucau
augcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugc
accuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAG
ACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCA
AACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAG
UAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCACAGCAUUGUACCCAGAGUC
UGUCCCCAGACAUUGCACCUGGCGCUGUGAGGGCAGUGUGUCGUAACGGGCAACUCU
GCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGC
UUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGA
CUAAUAGAGCUAUUAUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGU

UAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacauggucc ugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauaguc uagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCGGAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGUCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCGACAGCAUUGUACCCAGAGUCUGUCCCCAGACAUUGCACCUGGCGCUGUCCA**

UCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUG
AAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccguga
gcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccaca
aguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccac
cggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuac
cccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucu
ucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcau
cgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagc
cacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucg
aggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcc
cgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugc
uggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucaugcaucucg
agugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccu
cuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACG
GACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGG
GAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGU
AAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

MS2CP responsive A-cap Linear EGFP variant4

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGCAGGUGAGGAUCACCCAUCUGCCA
CGAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUUCGAUCGUUACCAUAUAGCUAUUG
GAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCAC
UUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggga

uccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccac
aaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggc
aagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacau
gaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggc
aacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuuc
aaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaag
cagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuacc
agcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaa
agaccccaacgagaagcgcgaucacauggucCugcuggaguucgugaccgccgccgggaucacucucggcauggacga
gcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuuc
uucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUAC
CGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAA

MS2CP responsive A-cap Linear EGFP variant5

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGAGCAGGUGAGGAUCACCCAUCUG
CCACGAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUUCUCCCUUUGUUGGGUUUAUA
CCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAa
ugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacg
gccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccac
cggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgacc

acaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacga
cggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucga
cuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccga
caagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgacca
cuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccuga
gcaaagaccccaacgagaagcgcgaucacauggucc ugcuggaguucgugaccgccgccgggaucacucucggcaugg
acgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcc
cuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGU
UACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAA

MS2CP responsive A-cap Linear EGFP variant6

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUU
AGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAGAGCAG
GUGAGGAUCACCCAUCUGCCACGAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUUCa
ugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacg
gccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccac
cggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgacc
acaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacga
cggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucga
cuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccga

caagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgacca
cuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccuga
gcaaagaccccaacgagaagcgcgaucacauggucugcuggaguucgugaccgccgccgggaucacucucggcaugg
acgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcc
cuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGCUAUUAUGCGU
UACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAA

U1A responsive A-cap Linear EGFP variant4

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCU
AGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGU
AACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAU
CAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAG
AAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCA
GAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCC
CACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGG
ACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGC
CCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGU
GUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUU
AUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCGACAGCAUUGUACCCAGAGUCUGUCCCCAGACAUUGCACCUGGCGCUGUCCAU
CCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAA
AGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagg
gcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcgugu
ccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcc
cuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgac
uucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagaccc
gcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggca
acauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggca
ucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccc
caucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgag
aagcgcgaucacauggucugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagaga

ucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

U1A responsive A-cap Linear EGFP variant5

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGCCGGCGAAUUAAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACCUUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGUAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGACAGCAUUGUACCCAGAGUCUGUCCCCAGACAUUGCACCUGGCGCUGUCUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAA

U1A responsive A-cap Linear EGFP variant6

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

GGGAAAAUCCGUUGACCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCC
GGAAACGCAAUAGCCGGCGAAUUAAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACAC
CGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGGU
AUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAGUA
ACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUUCUG
UUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUUCGUU
AUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGUUUCGCU
CAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGGGCGACCG
UGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCUCUAAUACA
GACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCUGAAUGCGGC
UAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUCGUAACGGGCA
ACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAUUCCUAUACUGG
CUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGAUUGGCCAUCCGG
UGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACUUAGCUUGAAAGAG
GUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAGACAGCAUUGUACCCAGA
GUCUGUCCCCAGACAUUGCACCUGGCGCUGUCaugggauccgugagcaagggcgaggagcuguucac
cgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguccggcgagggcgaggg
cgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccuggcccacccucgug
accacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgcca
ugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaagu
ucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcaca
agcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuuca
agauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccc
cgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaug
guccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucuc
gagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggu
cuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGCAGGUGAGGAUCACCCAUCUGCCAC
GAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUUCGAUCGUUACCAUAUAGCUAUUG
GAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACC
ACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAa
ugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacg
uaaacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguu
caucugcaccaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugc
uucagccgcuaccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccagg
agcgcaccaucuucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccu
ggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguac
aacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagaucc
gccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggcccc
gugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgauc
acaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucuc
auaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuu
gcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCG
GCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGC

UCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGA
AGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGA
AACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUC
CCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGACAGCAUUGUACCCAGAGUCUG
UCCCCAGACAUUGCACCUGGCGCUGUCUCCCUUUGUUGGGUUUAUACCACUUAGCUU
GAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgug
agcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccac
aaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcacca
ccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcua
ccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccauc
uucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgca
ucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacag
ccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacauc
gaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugc
ccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccug
cuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucuc

gagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguacc
ucuuggucuuuGAAUAAAGCCUGAGUAGGGGCUAUUAUGCGUUACCGGCGAGACGCUAC
GGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAG
GGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAG
UAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGAC
CAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCG
AGCUCGAAUU

U1A responsive Circular EGFP +pA variant5 (**bold: circularized**)
Group I intron, CVB3 IRES, EGFP, U1A binding motif
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGACAGCAUUGUACCCAGAGUCUG
UCCCCAGACAUUGCACCUGGCGCUGUCUCCCUUUGUUGGGUUUAUACCACUUAGCUU
GAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugggauccgug
agcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccac
aaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcacca
ccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcua
ccccgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccauc
uucuucaaggacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgca
ucgagcugaagggcaucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacag
ccacaacgucuauaucauggccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacauc

gaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAG

Cap-MS2CP

MS2CP

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUcgccaccaugGCUUCUAACUUUACUCAGUUCGUUCUCGUCGACAAUGGCGGAACUGGCGACGUGACUGUCGCCCCAAGCAACUUCGCUAACGGGGUCGCUGAAUGGAUCAGCUCUAACUCGCGAUCACAGGCUUACAAAGUAACCUGUAGCGUUCGUCAGAGCUCUGCGCAGAAUCGCAAAUACACCAUCAAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUUACUUAAAUAUGGAACUAACCAUUCCAAUUUUCGCCACGAAUUCCGACUGCGAGCUUAUUGUUAAGGCAAUGCAAGGUCUCCUAAAAGAUGGAAACCCGAUUCCCUCGGCCAUCGCGGCCAACUCCGGCAUCUACUGAucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Cap-U1A

U1A

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUcgccaccAUGGcgGCAGUUCCCGAGACCCGCCCUAACCACACUAUUUAUAUCAACAACCUCAAUGAGAAGAUCAAGAAGGAUGAGCUAAAAAAGUCCCUGUACGCCAUCUUCUCCCAGUUUGGCCAGAUCCUGGAUAUCCUGGUAUCACGGAGCCUGAAGAUGAGGGGCCAGGCCUUUGUCAUCUUCAAGGAGGUCAGCAGCGCCACCAACGCCCUGCGCUCCAUGCAGGGUUUCCCUUUCUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCAGAUAUCAUUGCCAAGAUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGAGGAAGCCCAAGAGCCAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGGAGCCACCCCCGUGGUGGGGGCUGUCCAGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUCAGGCGCCCCGCAUUAUGCACCAC

AUGCCGGGCCAGCCGCCCUACAUGCCGCCCCCUGGUAUGAUCCCCCGCCAGGCCUUG
CACCUGGCCAGAUCCCACCAGGGGCCAUGCCCCCGCAGCAGCUUAUGCCAGGACAGAU
GCCCCCUGCCCAGCCUCUUUCUGAGAAUCCACCGAAUCACAUCUUGUUCCUCACCAACC
UGCCAGAGGAGACCAACGAGCUCAUGCUGUCCAUGCUUUUCAAUCAGUUCCCUGGCUU
CAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUCGCCUUCGUGGAGUUUGACAAU
GAGGUACAGGCAGGGGCAGCUCGCGAUGCCCUGCAGGGCUUUAAGAUCACGCAGAACA
ACGCCAUGAAGAUCUCCUUUGCCAAGAAGUAGucuagaccuucugcggggcuugccuucuggccaugc
ccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugG
CUUCUAACUUUACUCAGUUCGUUCUCGUCGACAAUGGCGGAACUGGCGACGUGACUG
UCGCCCCAAGCAACUUCGCUAACGGGGUCGCUGAAUGGAUCAGCUCUAACUCGCGAU
CACAGGCUUACAAAGUAACCUGUAGCGUUCGUCAGAGCUCUGCGCAGAAUCGCAAAU
ACACCAUCAAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUUACUUAAAUAUGGAAC

UAACCAUUCCAAUUUUCGCCACGAAUUCCGACUGCGAGCUUAUUGUUAAGGCAAUGCA
AGGUCUCCUAAAAGAUGGAAACCCGAUUCCCUCGGCCAUCGCGGCCAACUCCGGCAU
CUACUGAAGCAAGUACAUCCACGUUUAAGUucuagaccuucugcggggcuugccuucuggccau
gcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGC
UAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAA
UUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAA
UCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGC
AUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGG
UCGACUCUAGAGGAUC

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

Group I intron, CVB3 IRES, miR-302a-5p target site, U1A
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAAUGG
cgGCAGUUCCCGAGACCCGCCCUAACCACACUAUUUAUAUCAACAACCUCAAUGAGAA
GAUCAAGAAGGAUGAGCUAAAAAAGUCCCUGUACGCCAUCUUCUCCCAGUUUGGCCA
GAUCCUGGAUAUCCUGGUAUCACGGAGCCUGAAGAUGAGGGGCCAGGCCUUUGUCAU
CUUCAAGGAGGUCAGCAGCGCCACCAACGCCCUGCGCUCCAUGCAGGGUUUCCCUUU

**CUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCAGAUAUCAUUGCCAAG
AUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGAGGAAGCCCAAGAGC
CAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGGAGCCACCCCCGUGGUG
GGGGCUGUCCAGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUCAGGCGCCCCGCAUU
AUGCACCACAUGCCGGGCCAGCCGCCCUACAUGCCGCCCCCUGGUAUGAUCCCCCG
CCAGGCCUUGCACCUGGCCAGAUCCCACCAGGGGCCAUGCCCCCGCAGCAGCUUAUG
CCAGGACAGAUGCCCCCUGCCCAGCCUCUUUCUGAGAAUCCACCGAAUCACAUCUUGU
UCCUCACCAACCUGCCAGAGGAGACCAACGAGCUCAUGCUGUCCAUGCUUUUCAAUCA
GUUCCCUGGCUUCAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUCGCCUUCGU
GGAGUUUGACAAUGAGGUACAGGCAGGGGCAGCUCGCGAUGCCCUGCAGGGCUUUAA
GAUCACGCAGAACAACGCCAUGAAGAUCUCCUUUGCCAAGAAGUAGAGCAAGUACAU
CCACGUUUAAGUucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcacc
uguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGA
GACGCUACGGACUU**AAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUC
AAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUA
GUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACA
GCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUC

EGFP linear switch (MS2CP)

EGFP, MS2CP binding motif

GGUCAGAUCCGCUAGCGGAUCCGGGAGCAGGUGAGGAUCACCCAUCUGCCACGAGCGA
GGUGAGGAUCACCCAUCUCGCUCGUGUUCCCACCGGUCgccaccaugggauccgugagcaagggc
gaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacguaaacggccacaaguucagcguguc
ggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugcaccaccggcaagcugcccgugcccu
ggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccccgaccacaugaagcagcacgacuu
cuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaaggacgacggcaacuacaagacccgc
gccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggcaucgacuucaaggaggacggcaac
auccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucauggccgacaagcagaagaacggcauc
aaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgccgaccacuaccagcagaacacccccа
ucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgcccugagcaaagaccccaacgagaa
gcgcgaucacauggucсugcuggaguucgugaccgccgccgggaucacucucggcauggacgagcuguacaagagauc
ucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcacc
uguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

AAAAAAAAAAAAAAAAAAAAAAAAAAA

EGFP linear switch (U1A)

EGFP, U1A binding motif

GACAGCAUUGUACCCAGAGUCUGUCCCCAGACAUUGCACCUGGCGCUGUCCGCAGAUC
GAGAAGAAGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUgcc
accaugggauccgugagcaagggcgaggagcuguucaccgggguggugcccauccuggucgagcuggacggcgacgua
aacggccacaaguucagcguguccggcgagggcgagggcgaugccaccuacggcaagcugacccugaaguucaucugc
accaccggcaagcugcccgugcccuggcccacccucgugaccacccugaccuacggcgugcagugcuucagccgcuaccc
cgaccacaugaagcagcacgacuucuucaaguccgccaugcccgaaggcuacguccaggagcgcaccaucuucuucaag
gacgacggcaacuacaagacccgcgccgaggugaaguucgagggcgacacccuggugaaccgcaucgagcugaagggc
aucgacuucaaggaggacggcaacauccuggggcacaagcuggaguacaacuacaacagccacaacgucuauaucaug
gccgacaagcagaagaacggcaucaaggugaacuucaagauccgccacaacaucgaggacggcagcgugcagcucgcc
gaccacuaccagcagaacacccccaucggcgacggccccgugcugcugcccgacaaccacuaccugagcacccaguccgc
ccugagcaaagaccccaacgagaagcgcgaucacaugguccugcuggaguucgugaccgccgccgggaucacucucggc
auggacgagcuguacaagagaucucauaugcaucucgagugauagucuagaccuucugcggggcuugccuucuggcca
ugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC

GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGCAGGUGAGGAUCACCCAUCUGCCAC
GAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUUCGAUCGUUACCAUAUAGCUAUUG
GAUUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACC
ACUUAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAa
uggacaucaagguggguucacccugguguucagcgcccuggugcaggccaagagcaccgaguucgacccca
acaucgacaucgugggccuggaaggcaaguucggcaucaccaaccuggaaaccgaccuguucaccaucuggga
gaccauggaagugaugaucaaggccgacaucgccgacaccgaccgggccagcaacuucguggccaccgagacc
gacgccaaccggggcaagaugcccggcaagaagcugccccuggccgucaucauggaaauggaagccaacgccu
ucaaggccggcugcacccggggcugccugaucugccugagcaagaucaagugcaccgccaagaugaaggugua
cauccccggcaggugccacgacuacggcggcgacaagaaaaccggccaggccggcaucgugggcgccaucgug
gacauccccgagaucagcggcuucaaagaaauggcccccauggaacaguucaucgcccagguggacagaugcg
ccagcugcaccaccggcugccugaagggccuggccaacgugaagugcagcgagcugcugaagaaguggcugcc
cgaccgcugcgccagcuucgccgacaagauccagaaagaggugcacaacaucaagggcauggccggcgacagg
ugaucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuugg
ucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGG
ACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGG
AAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUA
AGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCA
UGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAG
CUCGAAUU

U1A responsive Circular MetLuc2 +pA variant5 (**bold: circularized**)
Group I intron, CVB3 IRES, MetLuc2, U1A binding motif
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG
UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU

UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUAGACAGCAUUGUACCCAGAGUCUG
UCCCCAGACAUUGCACCUGGCGCUGUCUCCCUUUGUUGGGUUUAUACCACUUAGCUU
GAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAauggacaucaag
gugguguucacccugguguucagcgcccuggugcaggccaagagcaccgaguucgaccccaacaucgacaucg
ugggccuggaaggcaaguucggcaucaccaaccuggaaaccgaccuguucaccaucugggagaccauggaagu
gaugaucaaggccgacaucgccgacaccgaccgggccagcaacuucguggccaccgagaccgacgccaaccgg
ggcaagaugcccggcaagaagcugccccuggccgucaucauggaaauggaagccaacgccuucaaggccggcu
gcacccggggcugccugaucugccugagcaagaucaagugcaccgccaagaugaagguguacaucccggcag
gugccacgacuacggcggcgacaagaaaaccggccaggccggcaucgugggcgccaucguggacaucccgag
aucagcggcuucaaagaaauggcccccauggaacaguucaucgcccagguggacagaugcgccagcugcacca
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ccagcuucgccgacaagauccagaaagaggugcacaacaucaagggcauggccggcgacagguga ucuagacc
uucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUA
AAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUA
AUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAA
UCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUG
GACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGC
CAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCCGGGUACCGAGCUCGAAUU

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

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

GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG

UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAaugG
CUUCUAACUUUACUCAGUUCGUUCUCGUCGACAAUGGCGGAACUGGCGACGUGACUG
UCGCCCCAAGCAACUUCGCUAACGGGGUCGCUGAAUGGAUCAGCUCUAACUCGCGAU
CACAGGCUUACAAAGUAACCUGUAGCGUUCGUCAGAGCUCUGCGCAGAAUCGCAAAU
ACACCAUCAAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUUACUUAAAUAUGGAAC
UAACCAUUCCAAUUUUCGCCACGAAUUCCGACUGCGAGCUUAUUGUUAAGGCAAUGCA
AGGUCUCCUAAAAGAUGGAAACCCGAUUCCCUCGGCCAUCGCGGCCAACUCCGGCAU
CUACUGAUCAACAUCAGUCUGAUAAGCUAucuagaccuucugcggggcuugccuucuggccaugc
ccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCUA
UUAUGCGUUACCGGCGAGACGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUU
CUUUAAGUGGAUGCUCUCAAACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUC
CUGAGCCAAGCCGAAGUAGUAAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAU
AUCUAGACACAGGAAACAGCUAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUC
GACUCUAGAGGAUC

3’T21-5p Circular U1A +pA (**bold: circularized**)
Group I intron, CVB3 IRES, miR-21-5p target site, U1A
GGGAGACCCUCGACCGUCGAUUGUCCACUGGUCAACAAUAGAUGACUUACAACUAAUCG
GAAGGUGCAGAGACUCGACGGGAGCUACCCUAACGUCAAGACGAGGGUAAAGAGAG
UCCAAUUCUCAAAGCCAAUAGGCAGUAGCGAAAGCUGCAAGAGAAUG**AAAAUCCGUUGA**
CCUUAAACGGUCGUGUGGGUUCAAGUCCCUCCACCCCCACGCCGGAAACGCAAUAGC
CGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC
UUAAAACAGCCUGUGGGUUGAUCCCACCCACAGGCCCAUUGGGCGCUAGCACUCUGG
UAUCACGGUACCUUUGUGCGCCUGUUUUAUACCCCCUCCCCCAACUGUAACUUAGAAG

UAACACACACCGAUCAACAGUCAGCGUGGCACACCAGCCACGUUUUGAUCAAGCACUU
CUGUUACCCCGGACUGAGUAUCAAUAGACUGCUCACGCGGUUGAAGGAGAAAGCGUU
CGUUAUCCGGCCAACUACUUCGAAAAACCUAGUAACACCGUGGAAGUUGCAGAGUGU
UUCGCUCAGCACUACCCCAGUGUAGAUCAGGUCGAUGAGUCACCGCAUUCCCCACGG
GCGACCGUGGCGGUGGCUGCGUUGGCGGCCUGCCCAUGGGGAAACCCAUGGGACGCU
CUAAUACAGACAUGGUGCGAAGAGUCUAUUGAGCUAGUUGGUAGUCCUCCGGCCCCU
GAAUGCGGCUAAUCCUAACUGCGGAGCACACACCCUCAAGCCAGAGGGCAGUGUGUC
GUAACGGGCAACUCUGCAGCGGAACCGACUACUUUGGGUGUCCGUGUUUCAUUUUAU
UCCUAUACUGGCUGCUUAUGGUGACAAUUGAGAGAUCGUUACCAUAUAGCUAUUGGA
UUGGCCAUCCGGUGACUAAUAGAGCUAUUAUAUCCCUUUGUUGGGUUUAUACCACU
UAGCUUGAAAGAGGUUAAAACAUUACAAUUCAUUGUUAAGUUGAAUACAGCAAAAUGG
cgGCAGUUCCCGAGACCCGCCCUAACCACACUAUUUAUAUCAACAACCUCAAUGAGAA
GAUCAAGAAGGAUGAGCUAAAAAAGUCCCUGUACGCCAUCUUCUCCCAGUUUGGCCA
GAUCCUGGAUAUCCUGGUAUCACGGAGCCUGAAGAUGAGGGGCCAGGCCUUUGUCAU
CUUCAAGGAGGUCAGCAGCGCCACCAACGCCCUGCGCUCCAUGCAGGGUUUCCCUUU
CUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCAGAUAUCAUUGCCAAG
AUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGAGGAAGCCCAAGAGC
CAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGGAGCCACCCCCGUGGUG
GGGGCUGUCCAGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUCAGGCGCCCCGCAUU
AUGCACCACAUGCCGGGCCAGCCGCCCUACAUGCCGCCCCCUGGUAUGAUCCCCCG
CCAGGCCUUGCACCUGGCCAGAUCCCACCAGGGGCCAUGCCCCCGCAGCAGCUUAUG
CCAGGACAGAUGCCCCCUGCCCAGCCUCUUUCUGAGAAUCCACCGAAUCACAUCUUGU
UCCUCACCAACCUGCCAGAGGAGACCAACGAGCUCAUGCUGUCCAUGCUUUUCAAUCA
GUUCCCUGGCUUCAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUCGCCUUCGU
GGAGUUUGACAAUGAGGUACAGGCAGGGGCAGCUCGCGAUGCCCUGCAGGGCUUUAA
GAUCACGCAGAACAACGCCAUGAAGAUCUCCUUUGCCAAGAAGUAGUCAACAUCAGUC
UGAUAAGCUAucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccugu
accucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGGCUAUUAUGCGUUACCGGCGAGA
CGCUACGGACUUAAAUAAUUGAGCCUUAAAGAAGAAAUUCUUUAAGUGGAUGCUCUCAA
ACUCAGGGAAACCUAAAUCUAGUUAUAGACAAGGCAAUCCUGAGCCAAGCCGAAGUAGU
AAUUAGUAAGACCAGUGGACAAUCGACGGAUAACAGCAUAUCUAGACACAGGAAACAGC
UAUGACCAUGAUUACGCCAAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUC

5’T302a-5p MS2CP

MS2CP, miR-302a-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAAUAUAAGACACCGGUcAGCAAGUACAUCCACGUUUAAGUgccaccaugGCUUCUAACUUUACUCAGUUCGUUCUCGUCGACAAUGGCGGAACUGGCGACGUGACUGUCGCCCCAAGCAACUUCGCUAACGGGGUCGCUGAAUGGAUCAGCUCUAACUCGCGAUCACAGGCUUACAAAGUAACCUGUAGCGUUCGUCAGAGCUCUGCGCAGAAUCGCAAAUACACCAUCAAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUUACUUAAAUAUGGAACUAACCAUUCCAAUUUUCGCCACGAAUUCCGACUGCGAGCUUAUUGUUAAGGCAAUGCAAGGUCUCCUAAAAGAUGGAAACCCGAUUCCCUCGGCCAUCGCGGCCAACUCCGGCAUCUACUGAucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

5’T302a-5p U1A

U1A, miR-302a-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAAUAUAAGACACCGGUcAGCAAGUACAUCCACGUUUAAGUgccaccAUGGcgGCAGUUCCCGAGACCCGCCCUAACCACACUAUUUAUAUCAACAACCUCAAUGAGAAGAUCAAGAAGGAUGAGCUAAAAAGUCCCUGUACGCCAUCUUCUCCCAGUUUGGCCAGAUCCUGGAUAUCCUGGUAUCACGGAGCCUGAAGAUGAGGGCCAGGCCUUUGUCAUCUUCAAGGAGGUCAGCAGCGCCACCAACGCCCUGCGCUCCAUGCAGGGUUUCCCUUUCUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCAGAUAUCAUUGCCAAGAUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGAGGAAGCCCAAGAGCCAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGGAGCCACCCCCGUGGUGGGGGCUGUCCAGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUCAGGCGCCCCGCAUUAUGCACCACAUGCCGGGCCAGCCGCCCUACAUGCCGCCCCCUGGUAUGAUCCCCCCGCCAGGCCUUGCACCUGGCCAGAUCCCACCAGGGGCCAUGCCCCCGCAGCAGCUUAUGCCAGGACAGAUGCCCCCUGCCCAGCCUCUUUCUGAGAAUCCACCGAAUCACAUCUUGUUCCUCACCAACCUGCCAGAGGAGACCAACGAGCUCAUGCUGUCCAUGCUUUUCAAUCAGUUCCCUGGCUUCAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUCGCCUUCGUGGAGUUUGACAAUGAGGUACAGGCAGGGGCAGCUCGCGAUGCCCUGCAGGGCUUUAAGAUCACGCAGAACAACGCCAUGAAGAUCUCCUUUGCCAAGAAGUAGucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

5’T21-5p MS2CP

MS2CP, miR-21-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUcUCAACAUCA
GUCUGAUAAGCUAgccaccaugGCUUCUAACUUUACUCAGUUCGUUCUCGUCGACAAUGGC
GGAACUGGCGACGUGACUGUCGCCCCAAGCAACUUCGCUAACGGGGUCGCUGAAUGGA
UCAGCUCUAACUCGCGAUCACAGGCUUACAAAGUAACCUGUAGCGUUCGUCAGAGCUCU
GCGCAGAAUCGCAAAUACACCAUCAAAGUCGAGGUGCCUAAAGGCGCAUGGAGGUCUUA
CUUAAAUAUGGAACUAACCAUUCCAAUUUUCGCCACGAAUUCCGACUGCGAGCUUAUUG
UUAAGGCAAUGCAAGGUCUCCUAAAAGAUGGAAACCCGAUUCCCUCGGCCAUCGCGGCC
AACUCCGGCAUCUACUGAucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugca
ccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAA

5’T21-5p U1A

U1A, miR-21-5p target site

GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUcUCAACAUCA
GUCUGAUAAGCUAgccaccAUGGcgGCAGUUCCCGAGACCCGCCCUAACCACACUAUUUAU
AUCAACAACCUCAAUGAGAAGAUCAAGAAGGAUGAGCUAAAAAAGUCCCUGUACGCCAU
CUUCUCCCAGUUUGGCCAGAUCCUGGAUAUCCUGGUAUCACGGAGCCUGAAGAUGAGG
GGCCAGGCCUUUGUCAUCUUCAAGGAGGUCAGCAGCGCCACCAACGCCCUGCGCUCCA
UGCAGGGUUUCCCUUUCUAUGACAAACCUAUGCGUAUCCAGUAUGCCAAGACCGACUCA
GAUAUCAUUGCCAAGAUGAAAGGCACCUUCGUGGAGCGGGACCGCAAGCGGGAGAAGA
GGAAGCCCAAGAGCCAGGAGACCCCGGCCACCAAGAAGGCUGUGCAAGGCGGGGGAGC
CACCCCCGUGGUGGGGGCUGUCCAGGGGCCUGUCCCGGGCAUGCCGCCGAUGACUCA
GGCGCCCCGCAUUAUGCACCACAUGCCGGGCCAGCCGCCCUACAUGCCGCCCCCUGGU
AUGAUCCCCCCGCCAGGCCUUGCACCUGGCCAGAUCCCACCAGGGGCCAUGCCCCCGC
AGCAGCUUAUGCCAGGACAGAUGCCCCCUGCCCAGCCUCUUUCUGAGAAUCCACCGAAU
CACAUCUUGUUCCUCACCAACCUGCCAGAGGAGACCAACGAGCUCAUGCUGUCCAUGCU
UUUCAAUCAGUUCCCUGGCUUCAAGGAGGUCCGUCUGGUACCCGGGCGGCAUGACAUC
GCCUUCGUGGAGUUUGACAAUGAGGUACAGGCAGGGGCAGCUCGCGAUGCCCUGCAGG
GCUUUAAGAUCACGCAGAACAACGCCAUGAAGAUCUCCUUUGCCAAGAAGUAGucuagacc
uucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCC
UGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAA

MetLuc2 linear switch (MS2CP)

MetLuc2, MS2CP binding motif

GGUCAGAUCCGCUAGCGGAUCCGGGAGCAGGUGAGGAUCACCCAUCUGCCACGAGCGA
GGUGAGGAUCACCCAUCUCGCUCGUGUUCCCACCGGUCgccaccauggacaucaagguggguguuc
acccugguguucagcgcccuggugcaggccaagagcaccgaguucgaccccaacaucgacaucgugggccuggaaggca
aguucggcaucaccaaccuggaaaccgaccuguucaccaucugggagaccauggaagugaugaucaaggccgacaucg
ccgacaccgaccgggccagcaacuucguggccaccgagaccgacgccaaccggggcaagaugcccggcaagaagcugcc
ccuggccgucaucauggaaauggaagccaacgccuucaaggccggcugcacccggggcugccugaucugccugagcaag
aucaagugcaccgccaagaugaagguguacauccccggcaggugccacgacuacggcggcgacaagaaaaccggccag
gccggcaucgugggcgccaucguggacauccccgagaucagcggcuucaaagaaauggcccccauggaacaguucauc
gcccagguggacagaugcgccagcugcaccaccggcugccugaagggccuggccaacgugaagugcagcgagcugcug
aagaaguggcugcccgaccgcugcgccagcuucgccgacaagauccagaaagaggugcacaacaucaagggcauggcc
ggcgacaggugaucuagaccuucugcggggcuugccuucuggccaugcccuucuucucucccuugcaccuguaccucuu
ggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAA

MetLuc2 linear switch (U1A)

MetLuc2, U1A binding motif

GACAGCAUUGUACCCAGAGUCUGUCCCCAGACAUUGCACCUGGCGCUGUCCGCAGAUC
GAGAAGAAGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUgcc
accauggacaucaagguggguguucacccugguguucagcgcccuggugcaggccaagagcaccgaguucgaccccaac
aucgacaucgugggccuggaaggcaaguucggcaucaccaaccuggaaaccgaccuguucaccaucugggagaccaug
gaagugaugaucaaggccgacaucgccgacaccgaccgggccagcaacuucguggccaccgagaccgacgccaaccggg
gcaagaugcccggcaagaagcugccccuggccgucaucauggaaauggaagccaacgccuucaaggccggcugcacccg
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cggcggcgacaagaaaaccggccaggccggcaucgugggcgccaucguggacauccccgagaucagcggcuucaaaga
aauggcccccauggaacaguucaucgcccagguggacagaugcgccagcugcaccaccggcugccugaagggccuggcc
aacgugaagugcagcgagcugcugaagaaguggcugcccgaccgcugcgccagcuucgccgacaagauccagaaagag
gugcacaacaucaagggcauggccggcgacaggugaucuagaccuucugcggggcuugccuucuggccaugcccuucu
ucucucccuugcaccuguaccucuuggucuuuGAAUAAAGCCUGAGUAGGAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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S1. Gale,M., Li,Y., Cao,J., Liu,Z. Z., Holmbeck,M.A., Zhang,M., Lang,S.M., Wu,L., Carmo,M.D., Gupta,S. *et al.* (2020) Acquired resistance to HER2-targeted therapies creates vulnerability to ATP synthase inhibition. *Cancer Res.* **80**, 524–535.

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S6. Kawasaki,S., Fujita,Y., Nagaike,T., Tomita,K. and Saito,H. (2017) Synthetic mRNA devices that detect endogenous proteins and distinguish mammalian cells. *Nucleic Acids Res.* **45**, e117.