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Supporting Information

VPg-Based Bidirectional Synthetic mRNA Circuits Enable Orthogonal Protein Regulation For High-Resolution Cell Separation

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MATERIAL AND METHODS

Cell culture

HEK293, HeLa, and HepG2-RFP were cultured in 89% Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum and 1% non-essential amino acids (Sigma-Aldrich). SK-BR-3 was cultured in Rosewell Park Memorial Institute 1640 Medium (ATCC 30-2001) with 10% Fetal Bovine Serum. HepG2/RFP cells were prepared by transduction with ready-to-use RNA encoding lentivirus particles for the cell culture system (Amsbio LVP428) according to the manufacturer's protocol. HepG2-RFP cells were sorted with BD FACSAria™ III (BD Biosciences) and selected by Gibco Geneticin (G418, Gibco). The cell culture is supplemented with 400 µg/mL G418 in addition to the mentioned culture medium to select the RFP-positive cell. All the cells were maintained at 37 °C with 5% CO_2. The cells were passaged when the confluency was 90%. After trypsinized for 3 minutes, the detached cells were suspended with 5× volume of culture medium and seeded at a density of 1.0×10^5 cells/mL.

The Human iPSC 201B7 cell line was maintained on the iMatrix-511 (Nippi. Inc) coating in the complete StemFit medium (Ajinomoto AK02N). The neural progenitor cell (NPC) was differentiated from the iPSC 201B7 cell line using Gibco PSC Neural Induction Medium (Gibco A1647801) following the manufacturer's instructions.

DNA template generation for *in vitro* transcription

All 5' UTR, 3' UTR, and ORF sequences used in this project are listed in Table S1, Table S2, and Table S3. Primers were purchased from Integrated DNA Technologies and Ruibo Biotech (Guangzhou). All PCR reactions were performed using Q5® Hot Start High-Fidelity 2× Master Mix (NEB) following the manufacturer's protocol. After cloning the ORFs from plasmids, the plasmid DNAs were removed from the PCR product using DpnI (NEB). The 5'UTRs and 3'UTRs were assembled using primers. The full templates for IVT were synthesized by fusion PCR with the attachment of a T7 promotor sequence and purified using the QIAquick® PCR purification kit (Qiagen). The full templates in water were quantified by NanoVue spectrophotometer (GE Healthcare).

Synthesis and purification of mRNA

All RNAs were synthesized by *in vitro* transcription (IVT) using MEGAscript™ T7 transcription kit (Invitrogen) following the manufacturer's protocol. The reaction cocktail consists of 7.5 nM ATP, 7.5 nM UTP or UTP analogues, 7.5 nM CTP or 5-Methylcytidine-5'-Triphosphate (Trilink), 1.5 nM GTP, and 6 nM cap analog. Non-modified NTPs were included in the T7 transcription kit. UTP analogue options include N^1-methylpseudouridine-5'-Triphosphate (Trilink) and pseudouridine (Trilink). Cap analogue options include Anti-reverse cap analog (TriLink), Acap analog (NEB), $m^7G(5')ppp(5')G$ RNA cap structure analogue (NEB), and trimethylated cap analogue (JenaBioscience). TURBO DNase (Invitrogen) was added to the IVT mixture and incubated at 37 °C for 30 minutes to remove the DNA template. The RNA products were purified using the RNEasy®MiniElute cleanup kit (Qiagen) and treated with Antarctic phosphatase (NEB) at 37 °C for 30 minutes to remove triphosphate on the product RNAs. The quality of the final products was confirmed using urea-PAGE and Fragment Analyzer (Agilent Advanced Analytical Technologies Fragment Analyzer). The RNA product in water was quantified by NanoVue spectrophotometer (GE Healthcare). The purified mRNAs were aliquoted and stored at -20 °C until use.

Transfection

HEK293, SK-BR-3, and HeLa were seeded at 2.5×10^4 cells/well into a 48-well plate with 250 µL culture medium one day before the transfection. For co-cultured flow cytometry experiments, HEK293 and HepG2-RFP cell lines were seeded into 48-well plates in a ratio of 1:2 with 3×10^4 cells/well in 0.25 mL/well of the medium. 201B7 and NPC were seeded with 10 µM ROCK inhibitor Y27632 at 2.0×10^4 cells/well and 2.5×10^4 cells/well respectively. After 24 hours, the media were replaced with the complete growth medium without Y27632 before transfection.

mRNAs were transfected using Lipofectamine MessengerMAX (Invitrogen) according to the manufacturer's instructions. The amounts of mRNA used in the experiment are listed in Table S4. iRFP mRNA was used as transfection control for flow cytometry analysis. Ctrl mRNA (FLuc) was used to balance the transfection load with the MCP mRNA-transfected sample.

Flow cytometry and analysis

After transfection, the cells were maintained at 37 °C, 5% CO_2 for 24 hours before analysis. The cells were rinsed using warm PBS buffer and trypsinized (Invitrogen™) for 3 minutes. The detached cells were suspended in 250 µL of culture medium and filtered through a 300-micron nylon mesh filter. The fluorescence intensities of the cells were examined using Attune® Nxt (Invitrogen) or BD FACS Aria IIIU or III. EGFP signals were analyzed by excitation laser at 488 nm and emission filter at 530/30 nm. iRFP670 signals were detected by excitation laser at 637 nm and emission filter at 670/14 nm on the Attune Nxt, or by excitation laser at 633 nm and emission filter at 660/20 nm on the BD FACS Aria IIIU and III. mCherry signals were detected by excitation laser at 561 nm and emission filter at 610/20 nm on the BD FACS Aria IIIU and III. RFP signals were detected by excitation laser at 561 nm and emission filter at 582/15 nm on BD FACS Aria IIIU and III. BFP signals were detected by excitation laser at 405 nm and emission filter at 450/40 nm on BD FACS Aria III. The mean fluorescent intensities in the arbitrary unit were obtained from the machines directly. The arbitrary units of fluorescent intensity were calibrated using standardized fluorescent beads (Attune Performance Tracking Beads from Invitrogen or CS&T Research Beads from BD) according to the manufacturer's instructions. If the gating is not specified, only iRFP670 positive cells were filtered as positively transfected cells and analyzed. For experiments transfected with miRNA mimic, iRFP was used for normalization of the EGFP signals.

Cell viability assay

A 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution was prepared by dissolving MTT (Invitrogen™) in PBS followed by filtration through 0.2 µm filter. The cell viability assay was conducted for cell cultures in a 48-well plate. After transfection, the cells were maintained at 37 °C, 5% CO_2 for 24 hours before analysis. 25 µL of the MTT solution was added to each well and the mixture was incubated at 37 °C for 4 hours. The purple formazan was dissolved in 275 µL of 10% SDS in 0.01 M HCl overnight at 37 °C. The well with medium only was used as the blank and underwent the same procedure. The absorbance at 560 nm was measured using Microplate Reader Varioskan (Thermo Fisher Scientific). The absorbance of each sample was adjusted by subtracting that of the blank. The viability of the negative control with only the transfection reagent was set to 100%.

Luciferase assay

24 hours after transfection, the cell medium of each well was collected and stored at -80 °C before the luciferase assay. The Cypridina luciferase (CLuc) assay was conducted based on the protocol by the NEB CLuc assay kit and Wu et al.[1]. In detail, 2 µL of conditioned medium was reacted with 38 µL of CLuc solution (1 µM Cypridina luciferin [Nanolight™ Technology], 60 mM phosphate buffer pH 6.4, 0.3 M sodium ascorbate, and 20 mM Na_2SO_3, prepared freshly and preincubated at room temperature for 30 minutes) in a black 96-well plate. Each sample was assayed in duplicate, and the medium without transfection of Cluc mRNA was used as blank. The luminescence was detected using Microplate Reader Varioskan (Thermo Scientific) with 2 seconds of mixing at 300 rpm and 25 °C with 10 seconds of integration. The Gaussia luciferase (GLuc) assay was conducted based on the protocol by Tannous[2]. In detail, 10 µL of conditioned medium was reacted with 50 µL of GLuc assay solution (20 µM coelenterazine [Nanolight™ Technology], 5 mM NaCl in 1×PBS, prepared freshly and preincubated at room temperature for 30 minutes). The luminescence detection protocol was the same as that of the CLuc assay. The duplicated luminescence signal was averaged and adjusted by subtracting the blank before analysis.

Confocal imaging

Quantitative confocal imaging followed the standard operating manual of CLSM (Leica SP8 Confocal Microscope). Parameters were selected following live sample confocal imaging measurement guidance. HEK293 cells were seeded in the 35 mm confocal dishes (glass bottom dish, SPL) and allowed to stabilize for 24 hours before transfection of mRNA. The seeding density was 1.2×10^5 cells in 2 mL of medium. Fluorescence observation and measurement were performed 24 hours after transfection. 63× oil immersion objective was used. The images were generated as 2048×2048 pixels with scanning at 100 Hz. Sequential scanning was used for multi-fluorescent quantification. The laser excitation and emission detecting settings were consistent at least throughout one experiment. For the miRNA multi-tracker system, emission filters for each fluorophore were tested and adjusted to ensure minimum channel crosstalk and signal leakages. EBFP2 used the 405 nm laser for excitation and 410-472 nm for emission detection. EGFP used the 488 nm laser for excitation and 493-560 nm for emission detection. mCherry used the 552 nm laser for excitation and 578-642 nm for emission detection. miRFP670 and iRFP670 used the 638 nm laser for excitation and 640-670 nm for emission detection.

Image analysis

All image analysis was performed using FIJI (distribution of the ImageJ software, US National Institutes of Health, Bethesda, Maryland, USA). Cell images were extracted, enhanced, and saved in tiff format from microscopy raw data. The enhancement included the default "despeckle" effect and the brightness and contrast adjustment. The same enhancement parameters were applied in samples of the same experiment. The ratiometric images were generated using the Calculus Plus function and further enhanced for better visualization.

Quantification and statistical analysis

Statistical values including the exact number of repeats (N = 3) and statistical significance were reported in the figures. Statistical analysis was performed using Microsoft Excel 2016 with the following formula: "Average ()" for mean values and "STDEV ()" for standard deviations. The significance levels were calculated through single-factor analysis of variance (ANOVA) by Microsoft Excel 2016 and denoted as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

SUPPLEMENTARY FIGURES

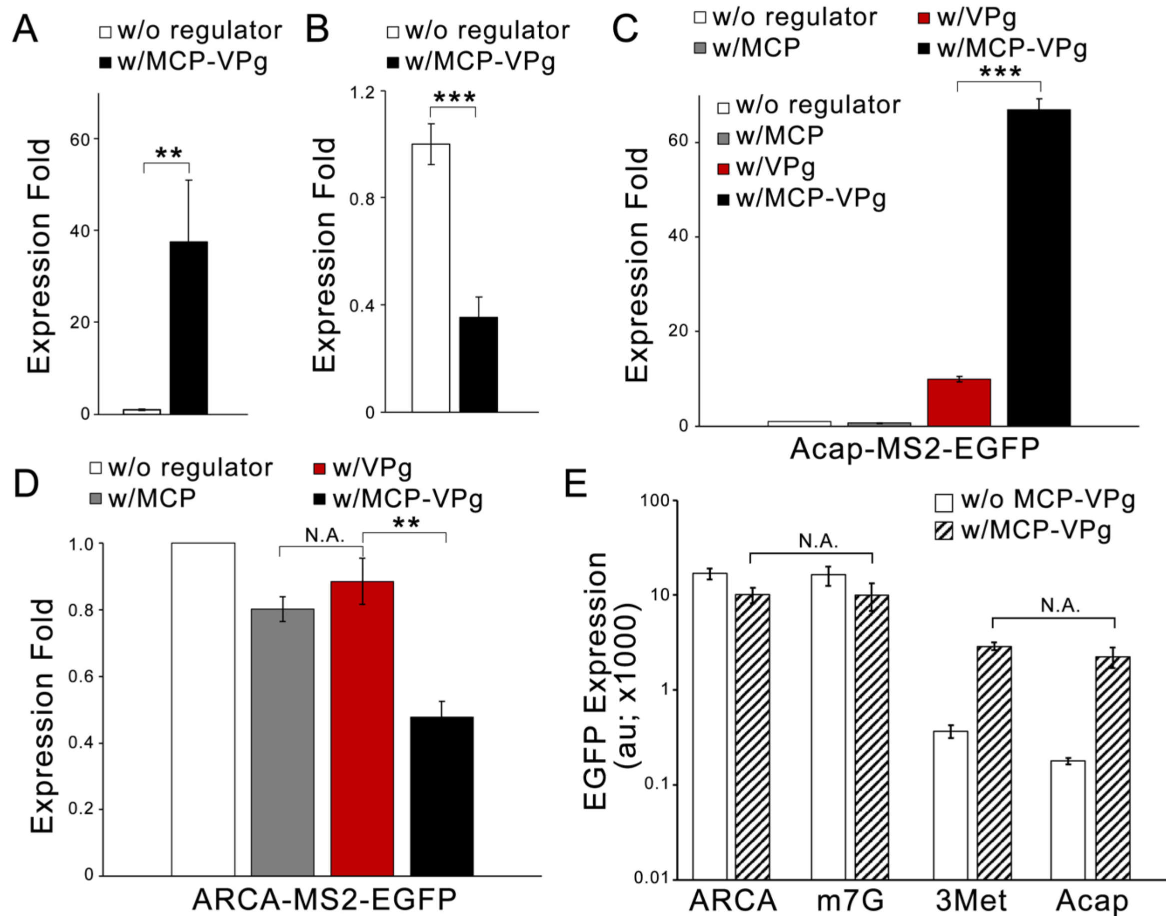


Figure S1 VPg overrides cap structures for translation initiation to produce opposite results on different mRNAs. (A) The bar graph showing the MCP-VPg-induced expression enhancement on Acap-MS2-EGFP on SK-BR-3 cells. The expression level of the reporter Acap-MS2-EGFP was set at 1. (B) The bar graph showing the MCP-VPg induced expression repression on ARCA-MS2-EGFP on SK-BR-3 cells. The expression level of the reporter ARCA-MS2-EGFP was set at 1. (C) The bar graph showing the effect of MCP, VPg, and MCP-VPg on Acap-MS2-EGFP on HEK293 cells. The expression level of the reporter Acap-MS2-EGFP was set at 1. (D) The bar graph showing the effect of MCP, VPg, and MCP-VPg on ARCA-MS2-EGFP on HEK293 cells. The expression level of the ARCA-MS2-EGFP was set at 1. (E) The expression levels of the MS2-EGFP mRNAs carrying different cap structures with or without cotransfection of MCP-VPg mRNA on HeLa cells. N=3; data are presented as mean ± SD with $^{**}P < 0.01$, $^{***}P < 0.001$ calculated through single-factor analysis of variance (ANOVA).

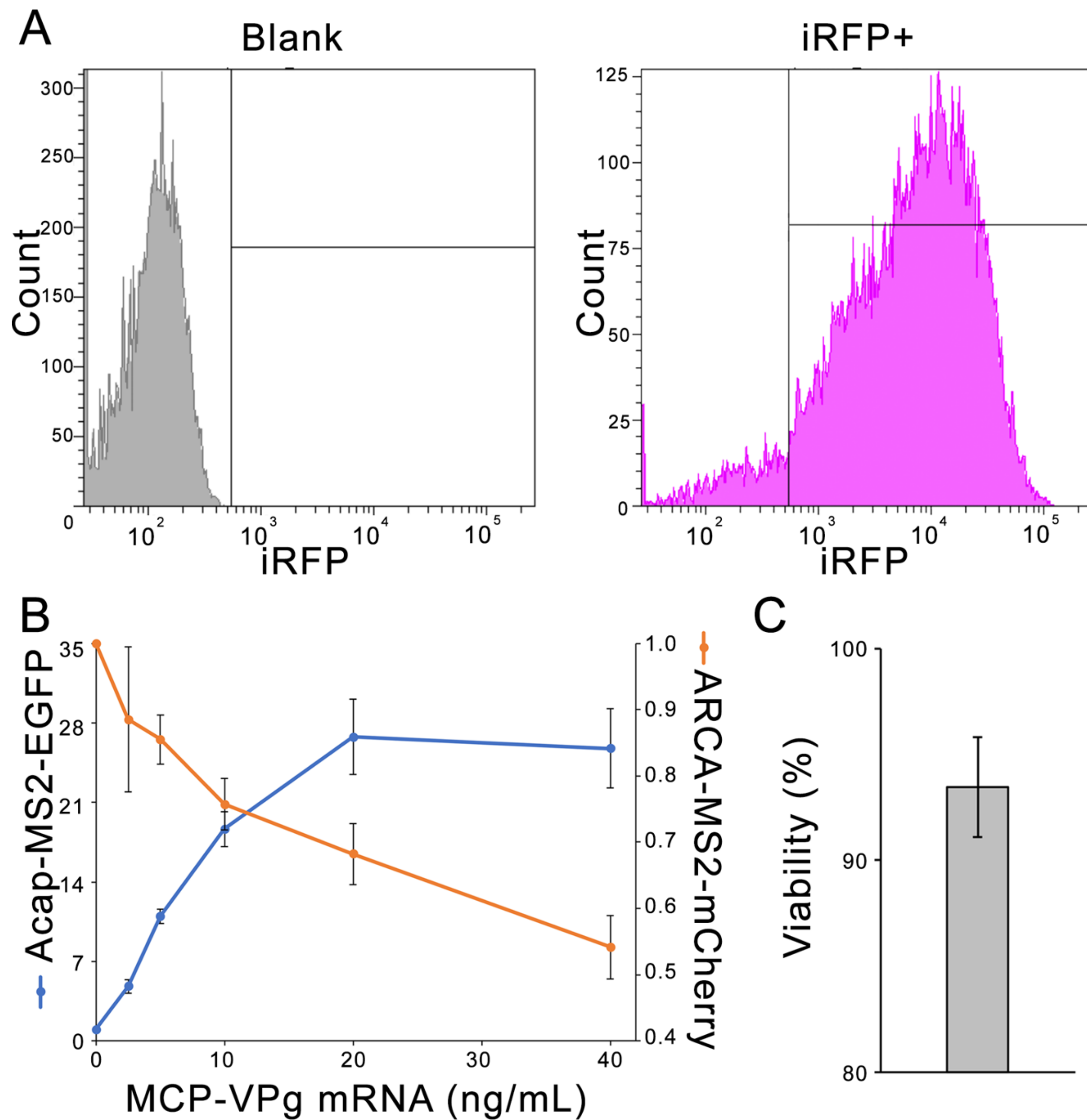


Figure S2 MCP-VPg mRNA-induced bidirectional output regulation on HEK293 cells. (A) The representative histograms showing the gating of positively transfected cells for further analysis. Left: Blank sample; Right: Transfected sample. (B) The relative expression levels showing MCP-VPg mRNA concentration-dependent orthogonal dual reporter expression on HEK293 cells. The expression levels of the Acap-MS2-EGFP and ARCA-MS2-mCherry mRNAs were set at 1. (C) The viability of the HEK293 cells at 24 hours after transfected with 40 ng/mL MCP-VPg, Acap-MS2-EGFP and ARCA-MS2-mCherry mRNAs. N=3; data are presented as mean ± SD.

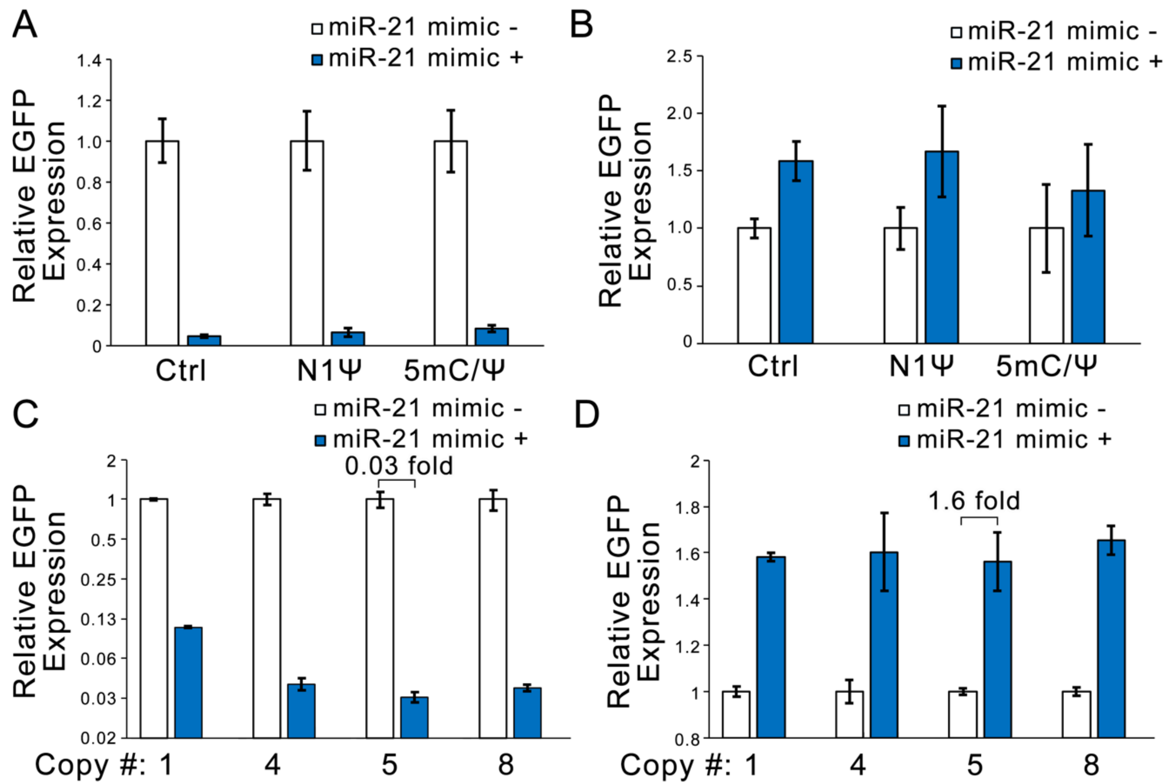
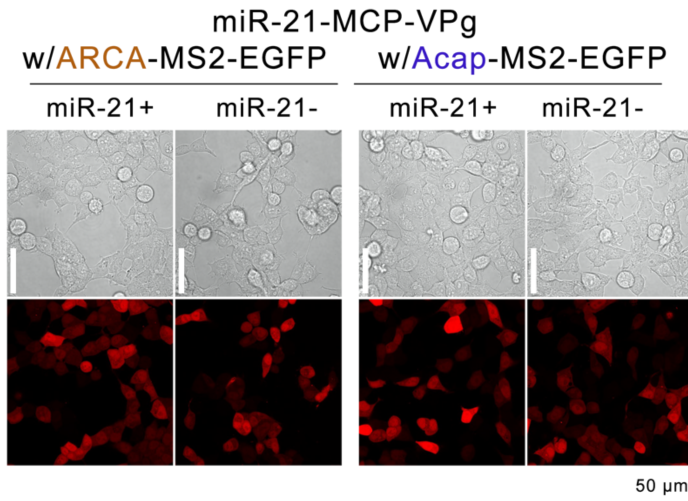


Figure S3 Optimization of the miR-21-sensing MCP-VPg mRNA. (A) The Acap-MS2-EGFP regulatory effect by miR-21-sensing MCP-VPg mRNA carrying different base modifications on HEK293 cells. The relative EGFP expression without miR-21 mimic was set at 1. (B) The ARCA-MS2-EGFP regulatory effect by miR-21-sensing MCP-VPg mRNA carrying different base modifications on HEK293 cells. The relative EGFP expression without miR-21 mimic was set at 1. (C) The Acap-MS2-EGFP regulatory effect by miR-21-sensing MCP-VPg mRNA carrying different copy numbers of miR-21 binding sites on HEK293 cells. The relative EGFP expression without miR-21 mimic was set at 1. (D) The ARCA-MS2-EGFP regulatory effect by miR-21-sensing MCP-VPg mRNA carrying different copy numbers of miR-21 binding sites on HEK293 cells. The relative EGFP expression without miR-21 mimic was set at 1. N=3; data are presented as mean ± SD.



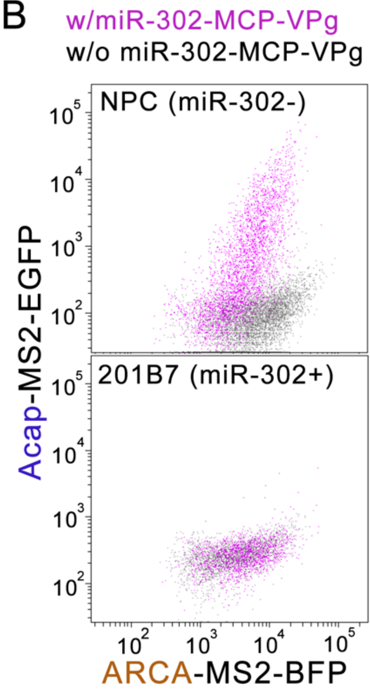


Figure S4 VPg-based bidirectional circuits afford high-resolution cell separation. (A) The representative confocal images showing the cell distribution in the bright field and the control iRFP signals on HEK293 cells after transfection of the miR-21 sensing circuit with or without miR-21 mimic. (B) The representative dot plots of EGFP and BFP expressions in NPC and 201B7 cells after transfection of the miR-302-sensing bidirectional circuit.

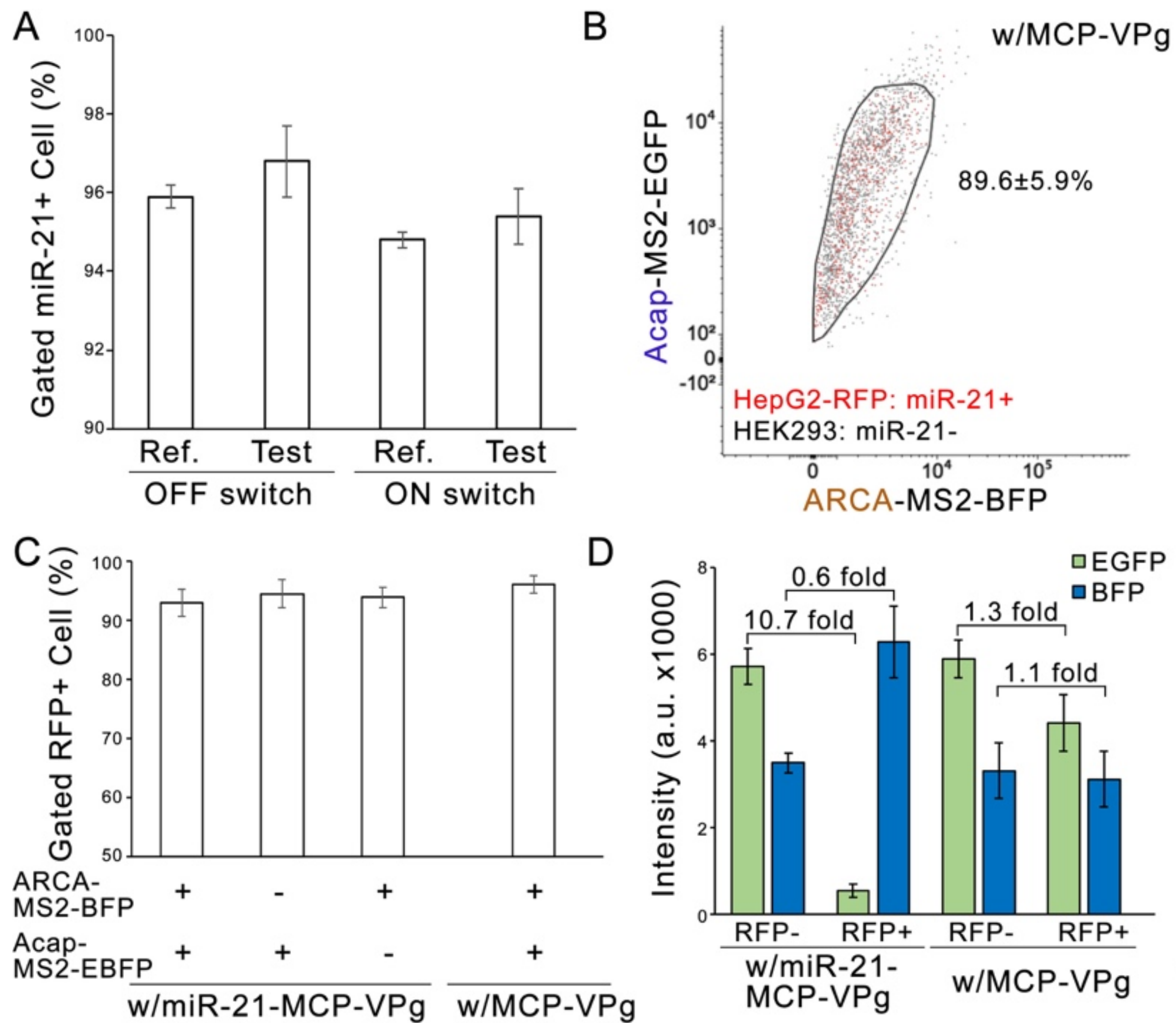


Figure S5 miR-controlled cell separation by the bidirectional circuit. (A) The bar graph showing the percentage of the gated miR-21 positive cells in Figure 2B and 2C, confirming the accuracy of gating. (B) The representative dot plot showing the performance of the miR-nonsensing circuit on the mixed culture of the HepG2-RFP and HEK293 cells. The percentages represent the HEK293 cells located in the gating of the HepG2 cell population. The high cell population overlap indicates the basal protein expression levels of the two cell lines were similar. (C) The bar graph showing the percentage of the gated HepG2-RFP cells in Figure 2D, confirming the accuracy of gating. (D) The bar graph showing the EGFP and BFP expressions of the two cell populations transfected with miR-sensing or miR-nonsensing circuits. N=3; data are presented as mean ± SD.

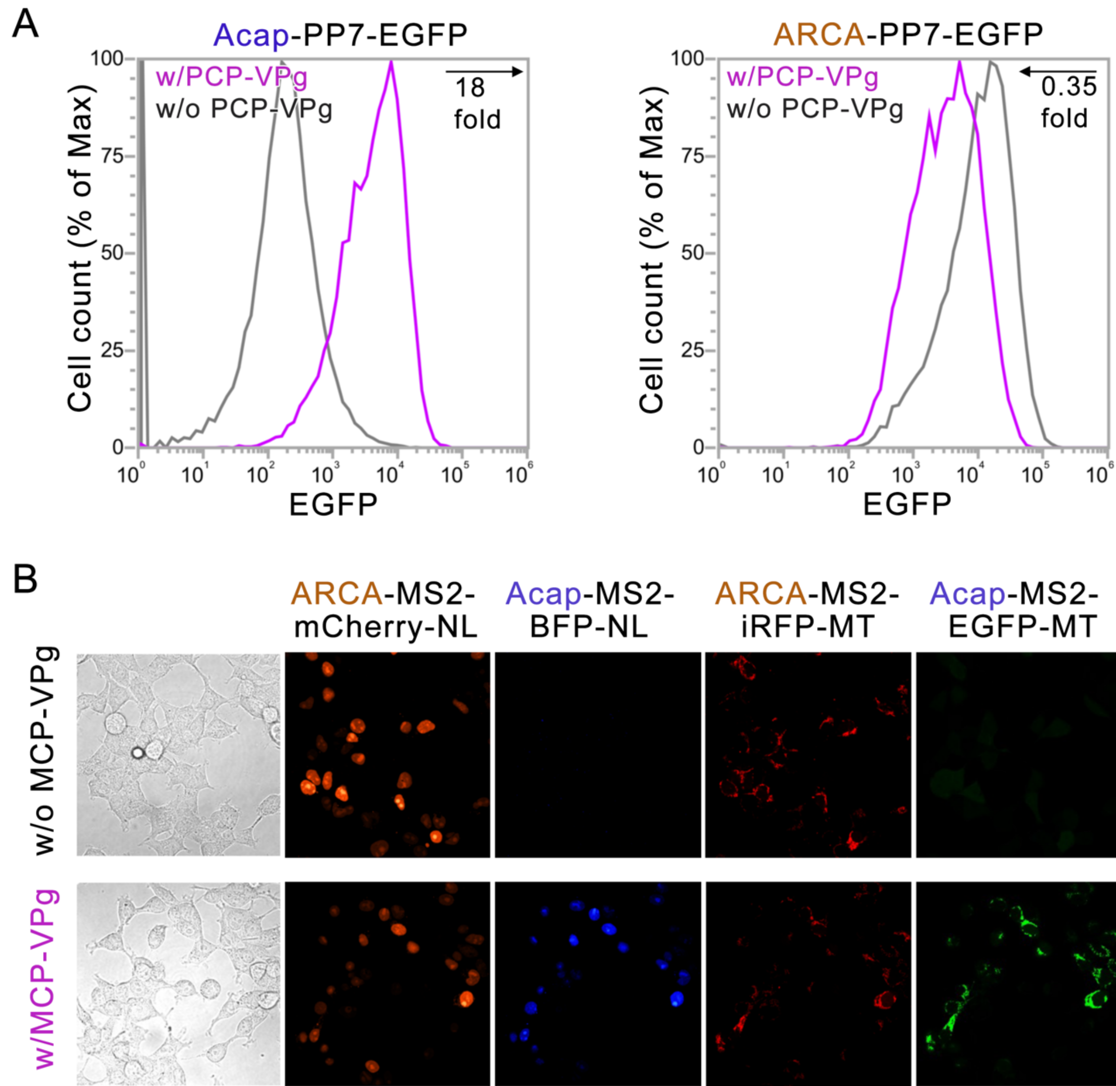


Figure S6 The VPg-based bidirectional circuit is flexible in design. (A) The representative histogram showing PCP-VPg-induced expression enhancement of Acap-PP7-EGFP and repression of ARCA-PP7-EGFP on HEK293 cells. The expression levels of Acap-PP7-EGFP and ARCA-PP7-EGFP were set at 1, respectively. N=3. (B) The representative low-magnification confocal images showing the bidirectional regulation of four fluorescent proteins on HEK293 cells. NL represents the nucleus tag and MT represents the mitochondrial tag.

SUPPLEMENTARY TABLES

Table S1. 5'UTRs used in this work

Name	Sequence (5'-3')	mRNAs containing this UTR sequence	Remark and Reference
5' UTR	GGGCGAAUUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGACACCGGUCGCCACC	MCP, VPg, MCP-VPg, PCP-VPg, iRFP670, Ctrl mRNA	[3]
MS2-5' UTR	GGGCUCAGAUCCGCUAGCGGAUGGUGAGGAUCACCCAUCUAUAAGACACCGGUCGCCACC	MS2-EGFP, MS2-mCherry, MS2-BFP, MS2-GLuc, MS2-CLuc, MS2-EGFP-MT, MS2-miRFP670-MT, MS2-BFP-NL, MS2-mCherry-NL	[4]
2×MS2-5'UTR	GGGUCAGAUCCGCUAGCGGAUCCGGGAGCAGGUGAGGAUCACCCAUCUGCCACGAGCGAGGUGAGGAUCACCCAUCUCGCUCGUGUUCCCACCGGUCGCCACC	2×MS2-PCP-VPg, 2×MS2-EGFP	[5]
PP7-5' UTR	GGGCUCAGAUCCGCUAGCGGAUUAAGGAGUUUAUAUGGAAACCCUUAUAUAAGACACCGGUCGCCACC	PP7-GFP, PP7-mCherry	[6]
1×anti-miR-21-5′ UTR	GGGCAUCGCGGAUCCUCAACAUCAGUCUGAUAAGCUAAGAUCACACCGGUCGCCACC	miR-21-MCP-VPg (1 copy)	[3]
4×anti-miR-21-5′ UTR	GGGCAUCCUCAACAUCAGUCUGAUAAGCUAUCAACAUCAGUCUGAUAAGCUAUCAACAUCAGUCUGAUAAGCUAUCAACAUCAGUC UGAUAAGCUAAGAUCACACCGGUCGCCACC	miR-21-MCP-VPg (4 copies, 5 copies, and 8 copies), miR-21-MCP, miR-21-EGFP	[3]
4×anti-miR-302-5′ UTR	GGGCAUCCAGCAAGUACAUCCACGUUUAAGUAGCAAGUACAUC CACGUUUAAGUAGCAAGUACAUCCACGUUUAAGUAGCAAGUAC AUCCACGUUUAAGUAGAUCACACCGGUCGCCACC	miR-302-MCP-VPg	[3]

Table S2. 3'UTRs used in this work

Name	Sequence (5'-3')	mRNAs containing this UTR sequence	Remark and Reference
3' UTR	UCUAGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGG	MCP, VPg, MCP-VPg, PCP-VPg, iRFP670, MS2-EGFP, MS2-mCherry, MS2-BFP, MS2-GLuc, MS2-CLuc, PP7-GFP, PP7-mCherry, miR-21-MCP-VPg (1 copy, 4 copies), 2×MS2-EGFP, 2×MS2-PCP-VPg, Ctrl mRNA, MS2-EGFP-MT, MS2-miRFP670-MT, MS2-BFP-NL, MS2-mCherry-NL	[3]
1×anti-miR-21-3′ UTR	UCUAGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUCUCCCUCAACAUCAGUCUGAUAAGCUACUUGGUCUUUGAAUAAAGCCUGAGUAGG	miR-21-MCP-VPg (5 copies), miR-21-EGFP. miR-21-MCP	[5]
4×anti-miR-21-3′ UTR	UCUAGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUCUCCCUCAACAUCAGUCUGAUAAGCUAUCAACAUCAGUCUGAUAAGCUAUCAACAUCAGUCUGAUAAGCUAUCAACAUCAGUCUGAUAAGCUACUUGGUCUUUGAAUAAAGCCUGAGUAGG	miR-21-MCP-VPg (8 copies)	[5]
1×anti-miR-302-3′ UTR	UCUAGACCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUCUCCCAGCAAGUACAUCCACGUUUAAGUCUUGGUCUUUGAAUAAAGCCUGAGUAGG	anti-miR-302a-MCP-VPg	[5]

Table S3. ORFs used in this work

Name	Sequence (5'-3')	mRNAs containing this sequence	Remark and reference
MCP	AUGGCUUCUAACUUUACUCAGUUCGUU CUCGUCGACAAUGGCGGAACUGGCGAC GUGACUGUCGCCCCAAGCAACUUCGCU AACGGGGUCGCUGAAUGGAUCAGCUCU AACUCGCGAUCACAGGCUUACAAAGUA ACCUGUAGCGUUCGUCAGAGCUCUGCG CAGAAUCGCAAAUACACCAUCAAAGUC GAGGUGCCUAAAGGCGCAUGGAGGUCU UACUUAAAUAUGGAACUAACCAUUCCA AUUUUCGCCACGAAUUCCGACUGCGAG CUUAUUGUUAAGGCAAUGCAAGGUCUC CUAAAAGAUGGAAACCCGAUUCCCUCG GCCAUCGCGGCCAACUCCGGCAUCUAC UAG	MCP, miR-21-MCP	Gifted from Hirohide Saito lab in Kyoto University[5]
VPg	AUGGGAAAGAAGGGCAAGAACAAGAAG GGCCGGGGGCGACCCGGAGUCUUCAGA ACCCGUGGGCUCACGGAUGAGGAGUAC GAUGAAUUCAAGAAGCGCCGCGAGUCU AGGGGCGGCAAGUACUCCAUUGAUGAU UACCUCGCUGACCGCGAGCGAGAAGAA GAACUCCUGGAGCGGGACGAGGAGGAG GCUAUCUUCGGGGAUGGCUUCGGGUUG AAGGCCACCCGCCGUUCCCGCAAGGCA GAGAGAGCCAAACUGGGCCUGGUUUCU GGUGGCGACAUCCGCGCCCGCAAGCCG AUCGACUGGAAUGUGGUUGGCCCCUCC UGGGCUGACGAUGACCGCCAGGUCGAC UACGGCGAGAAGAUCAACUUUGAGUAG	VPg	Synthesized by GenScript with sequence from GenBank (Access No. KM102450.1 MNV-VPg)[7]
MCP-VPg	AUGGCUUCUAACUUUACUCAGUUCGUU CUCGUCGACAAUGGCGGAACUGGCGAC GUGACUGUCGCCCCAAGCAACUUCGCU AACGGGGUCGCUGAAUGGAUCAGCUCU AACUCGCGAUCACAGGCUUACAAAGUA ACCUGUAGCGUUCGUCAGAGCUCUGCG CAGAAUCGCAAAUACACCAUCAAAGUC GAGGUGCCUAAAGGCGCAUGGAGGUCU UACUUAAAUAUGGAACUAACCAUUCCA AUUUUCGCCACGAAUUCCGACUGCGAG CUUAUUGUUAAGGCAAUGCAAGGUCUC CUAAAAGAUGGAAACCCGAUUCCCUCG GCCAUCGCGGCCAACUCCGGCAUCUAC GGAAAGAAGGGCAAGAACAAGAAGGGC CGGGGGCGACCCGGAGUCUUCAGAACC CGUGGGCUCACGGAUGAGGAGUACGAU GAAUUCAAGAAGCGCCGCGAGUCUAGG GGCGGCAAGUACUCCAUUGAUGAUUAC CUCGCUGACCGCGAGCGAGAAGAAGAA CUCCUGGAGCGGGACGAGGAGGAGGCU AUCUUCGGGGAUGGCUUCGGGUUGAAG GCCACCCGCCGUUCCCGCAAGGCAGAG AGAGCCAAACUGGGCCUGGUUUCUGGU GGCGACAUCCGCGCCCGCAAGCCGAUC GACUGGAAUGUGGUUGGCCCCUCCUGG	MCP-VPg, miR-21-MCP-VPg (1 copy, 4 copies, 5 copies, 8 copies), miR-302-MCP-VPg	Direct fusion of MCP and VPg

	GCUGACGAUGACCGCCAGGUCGACUAC GGCGAGAAGAUCAACUUUGAGUAG		
PCP-VPg	AUGGCCAAAACCAUCGUUCUUGCGGUC GGCGAGGCUACUCGCACUCUGACUGAG AUCCAGUCCACCGCAGACCGUCAGAUC UUCGAAGAGAAGGUCGGGCCUCUGGUG GGUCGGCUGCGCCUCACGGCUUCGCUC CGUCAAAACGGAGCCAAGACCGCGUAU CGCGUCAACCUAAAACUGGAUCAGGCG GACGUCGUUGAUGCAUCCACCAGCGUC GCCGGCGAGCUUCCGAAAGUGCGCUAC ACUCAGGUAUGGUCGCACGACGUGACA AUCGUUGCGAAUAGCACCGAGGCCUCG CGCAAAUCGUUGUACGAUUUGACCAAG UCCCUCGUCGCGACCUCGCAGGUCGAA GAUCUUGUCGUCAACCUUGUGCCGCUG GGCCGUGGAAAGAAGGGCAAGAACAAG AAGGGCCGGGGGCGACCCGGAGUCUUC AGAACCCGUGGGCUCACGGAUGAGGAG UACGAUGAAUUCAAGAAGCGCCGCGAG UCUAGGGGCGGCAAGUACUCCAUUGAU GAUUACCUCGCUGACCGCGAGCGAGAA GAAGAACUCCUGGAGCGGGACGAGGAG GAGGCUAUCUUCGGGGAUGGCUUCGGG UUGAAGGCCACCCGCCGUUCCCGCAAG GCAGAGAGAGCCAAACUGGGCCUGGUU UCUGGUGGCGACAUCCGCGCCCGCAAG CCGAUCGACUGGAAUGUGGUUGGCCCC UCCUGGGCUGACGAUGACCGCCAGGUC GACUACGGCGAGAAGAUCAACUUUGAG UAG	PCP-VPg, 2×MS2-PCP-VPg	Direct fusion of PCP and VPg PCP sequence was cloned from Addgene plasmid #27548, a gift from Kathleen Collins[8]
EGFP	AUGGGAUCCGUGAGCAAGGGCGAGGAG CUGUUCACCGGGGUGGUGCCCAUCCUG GUCGAGCUGGACGGCGACGUAAACGGC CACAAGUUCAGCGUGUCCGGCGAGGGC GAGGGCGAUGCCACCUACGGCAAGCUG ACCCUGAAGUUCAUCUGCACCACCGGC AAGCUGCCCGUGCCCUGGCCCACCCUCG UGACCACCCUGACCUACGGCGUGCAGU GCUUCAGCCGCUACCCCGACCACAUGA AGCAGCACGACUUCUUCAAGUCCGCCA UGCCCGAAGGCUACGUCCAGGAGCGCA CCAUCUUCUUCAAGGACGACGGCAACU ACAAGACCCGCGCCGAGGUGAAGUUCG AGGGCGACACCCUGGUGAACCGCAUCG AGCUGAAGGGCAUCGACUUCAAGGAGG ACGGCAACAUCCUGGGGCACAAGCUGG AGUACAACUACAACAGCCACAACGUCU AUAUCAUGGCCGACAAGCAGAAGAACG GCAUCAAGGUGAACUUCAAGAUCCGCC ACAACAUCGAGGACGGCAGCGUGCAGC UCGCCGACCACUACCAGCAGAACACCCC CAUCGGCGACGGCCCCGUGCUGCUGCC CGACAACCACUACCUGAGCACCCAGUC CGCCCUGAGCAAAGACCCCAACGAGAA GCGCGAUCACAUGGUCCUGCUGGAGUU CGUGACCGCCGCCGGGAUCACUCUCGG	MS2-EGFP, miR-21-EGFP, 2×MS2-EGFP	Gifted from Hirohide Saito lab in Kyoto University[5]

	CAUGGACGAGCUGUACAAGAGAUCUCA UAUGCAUCUCGAGUGA		
mCherry	AUGGUGAGCAAGGGCGAGGAGGAUAAC AUGGCCAUCAUCAAGGAGUUCAUGCGC UUCAAGGUGCACAUGGAGGGCUCCGUG AACGGCCACGAGUUCGAGAUCGAGGGC GAGGGCGAGGGCCGCCCCUACGAGGGC ACCCAGACCGCCAAGCUGAAGGUGACC AAGGGUGGCCCCCUGCCCUUCGCCUGG GACAUCCUGUCCCCUCAGUUCAUGUAC GGCUCCAAGGCCUACGUGAAGCACCCC GCCGACAUCCCCGACUACUUGAAGCUG UCCUUCCCCGAGGGCUUCAAGUGGGAG CGCGUGAUGAACUUCGAGGACGGCGGC GUGGUGACCGUGACCCAGGACUCCUCC CUGCAGGACGGCGAGUUCAUCUACAAG GUGAAGCUGCGCGGCACCAACUUCCCC UCCGACGGCCCCGUAAUGCAGAAGAAG ACCAUGGGCUGGGAGGCCUCCUCCGAG CGGAUGUACCCCGAGGACGGCGCCCUG AAGGGCGAGAUCAAGCAGAGGCUGAAG CUGAAGGACGGCGGCCACUACGACGCU GAGGUCAAGACCACCUACAAGGCCAAG AAGCCCGUGCAGCUGCCCGGCGCCUAC AACGUCAACAUCAAGUUGGACAUCACC UCCCACAACGAGGACUACACCAUCGUG GAACAGUACGAACGCGCCGAGGGCCGC CACUCCACCGGCGGCAUGGACGAGCUG UACAAG	MS2-mCherry	Cloned from Addgene plasmid #55110 mCherry-Nucleus-7, a gift from Michael Davidson
BFP	AUGGUGAGCAAGGGCGAGGAGCUGUUC ACCGGGGUGGUGCCCAUCCUGGUCGAG CUGGACGGCGACGUAAACGGCCACAAG UUCAGCGUGAGGGGCGAGGGCGAGGGC GAUGCCACCAACGGCAAGCUGACCCUG AAGUUCAUCUGCACCACCGGCAAGCUG CCCGUGCCCUGGCCCACCCUCGUGACCA CCCUGAGCCACGGCGUGCAGUGCUUCG CCCGCUACCCCGACCACAUGAAGCAGC ACGACUUCUUCAAGUCCGCCAUGCCCG AAGGCUACGUCCAGGAGCGCACCAUCU UCUUCAAGGACGACGGCACCUACAAGA CCCGCGCCGAGGUGAAGUUCGAGGGCG ACACCCUGGUGAACCGCAUCGAGCUGA AGGGCGUCGACUUCAAGGAGGACGGCA ACAUCCUGGGGCACAAGCUGGAGUACA ACUUCAACAGCCACAACAUCUAUAUCA UGGCCGUCAAGCAGAAGAACGGCAUCA AGGUGAACUUCAAGAUCCGCCACAACG UGGAGGACGGCAGCGUGCAGCUCGCCG ACCACUACCAGCAGAACACCCCCAUCG GCGACGGCCCCGUGCUGCUGCCCGACA GCCACUACCUGAGCACCCAGUCCGUGC UGAGCAAAGACCCCAACGAGAAGCGCG AUCACAUGGUCCUGCUGGAGUUCCGCA CCGCCGCCGGGAUCACUCUCGGCAUGG ACGAGCUGUACAAGUAA	MS2-BFP	Cloned from Addgene plasmid #55248, a gift from Michael Davidson

iRFP670	AUGGCGCGUAAGGUCGAUCUCACCUCC UGCGAUCGCGAGCCGAUCCACAUCCCC GGCAGCAUUCAGCCGUGCGGCUGCCUG CUAGCCUGCGACGCGCAGGCGGUGCGG AUCACGCGCAUUACGGAAAAUGCCGGC GCGUUCUUUGGACGCGAAACUCCGCGG GUCGGUGAGCUACUCGCCGAUUACUUC GGCGAGACCGAAGCCCAUGCGCUGCGC AACGCACUGGCGCAGUCCUCCGAUCCA AAGCGACCGGCGCUGAUCUUCGGUUGG CGCGACGGCCUGACCGGCCGCACCUUC GACAUCUCACUGCAUCGCCAUGACGGU ACAUCGAUCAUCGAGUUCGAGCCUGCG GCGGCCGAACAGGCCGACAAUCCGCUG CGGCUGACGCGGCAGAUCAUCGCGCGC ACCAAAGAACUGAAGUCGCUCGAAGAG AUGGCCGCACGGGUGCCGCGCUAUCUG CAGGCGAUGCUCGGCUAUCACCGCGUG AUGUUGUACCGCUUCGCGGACGACGGC UCCGGGAUGGUGAUCGGCGAGGCGAAG CGCAGCGACCUCGAGAGCUUUCUCGGU CAGCACUUUCCGGCGUCGCUGGUCCCG CAGCAGGCGCGGCUACUGUACUUGAAG AACGCGAUCCGCGUGGUCUCGGAUUCG CGCGGCAUCAGCAGCCGGAUCGUGCCC GAGCACGACGCCUCCGGCGCCGCGCUC GAUCUGUCGUUCGCGCACCUGCGCAGC AUCUCGCCCUGCCAUCUCGAAUUUCUG CGGAACAUGGGCGUCAGCGCCUCGAUG UCGCUGUCGAUCAUCAUUGACGGCACG CUAUGGGGAUUGAUCAUCUGUCAUCAU UACGAGCCGCGUGCCGUGCCGAUGGCG CAGCGCGUCGCGGCCGAAAUGUUCGCC GACUUCUUAUCGCUGCACUUCACCGCC GCCCACCACCAACGCAGAUCCAAUCAG UCUUCAAAUUUUGGACCCAUGAAGGGA GGAAAUUUUGGAGGCAGAAGCUCUGGC CCCUAUGGCGGUGGAGGCCAAUACUUU GCAAAACCACGAAACCAAGGUGGCUAU GGCGGUUCCAGCAGCAGCAGUAGCUAU GGCAGUGGCAGAAGAUUUAGAUCUCAU AUGCAUCUCGAGUGA	iRFP	Gifted from Hirohide Saito lab in Kyoto University[5]
CLuc	AUGAAGACCUUAAUUCUUGCCGUUGCA UUAGUCUACUGCGCCACUGUUCAUUGC CAGGACUGUCCUUACGAACCUGAUCCA CCAAACACAGUUCCAACUUCCUGUGAA GCUAAAGAAGGAGAAUGUAUUGAUAGC AGCUGUGGCACCUGCACGAGAGACAUA CUAUCAGAUGGACUGUGUGAAAAUAAA CCAGGAAAAACAUGUUGCCGAAUGUGU CAGUAUGUAAUUGAAUGCAGAGUAGAG GCCGCAGGAUGGUUUAGAACAUUCUAU GGAAAGAGAUUCCAGUUCCAGGAACCU GGUACAUACGUGUUGGGUCAAGGAACC AAGGGCGGCGACUGGAAGGUGUCCAUC ACCCUGGAGAACCUGGAUGGAACCAAG GGGGCUGUGCUGACCAAGACAAGACUG GAAGUGGCUGGAGACAUCAUUGACAUC GCUCAAGCUACUGAGAAUCCCAUCACU	MS2-CLuc	Cloned from Addgene plasmid #53222, a gift from Thorsten Stiewe[9]

	GUAAACGGUGGAGCUGACCCUAUCAUC GCCAACCCGUACACCAUCGGCGAGGUC ACCAUCGCUGUUGUUGAGAUGCCAGGC UUCAACAUCACCGUCAUUGAGUUCUUC AAACUGAUCGUGAUCGACAUCCUCGGA GGAAGAUCUGUAAGAAUCGCCCCAGAC ACAGCAAACAAAGGAAUGAUCUCUGGC CUCUGUGGAGAUCUUAAAAUGAUGGAA GAUACAGACUUCACUUCAGAUCCAGAA CAACUCGCUAUUCAGCCUAAGAUCAAC CAGGAGUUUGACGGUUGUCCACUCUAU GGAAAUCCUGAUGACGUUGCAUACUGC AAAGGUCUUCUGGAGCCGUACAAGGAC AGCUGCCGCAACCCCAUCAACUUCUAC UACUACACCAUCUCCUGCGCCUUCGCCC GCUGUAUGGGUGGAGACGAGCGAGCCU CACACGUGCUGCUUGACUACAGGGAGA CGUGCGCUGCUCCCGAAACUAGAGGAA CCUGCGUUUUGUCUGGACAUACUUUCU ACGAUACAUUUGACAAAGCAAGAUACC AAUUCCAGGGUCCCUGCAAGGAGAUUC UUAUGGCCGCCGACUGUUUCUGGAACA CUUGGGAUGUGAAGGUUUCACACAGGA AUGUUGACUCUUACACUGAAGUAGAGA AAGUACGAAUCAGGAAACAAUCGACUG UAGUAGAACUCAUUGUUGAUGGAAAAC AGAUUCUGGUUGGAGGAGAAGCCGUGU CCGUCCCGUACAGCUCUCAGAACACUU CCAUCUACUGGCAAGAUGGUGACAUAC UGACUACAGCCAUCCUACCUGAAGCUC UGGUGGUCAAGUUCAACUUCAAGCAAC UGCUCGUCGUACAUAUUAGAGAUCCAU UCGAUGGUAAGACUUGCGGUAUUUGCG GUAACUACAACCAGGAUUUCAGUGAUG AUUCUUUUGAUGCUGAAGGAGCCUGUG AUCUGACCCCCAACCCACCGGGAUGCA CCGAAGAACAGAAACCUGAAGCUGAAC GACUCUGCAAUAGUCUCUUCGCCGGUC AAAGUGAUCUUGAUCAGAAAUGUAACG UGUGCCACAAGCCUGACCGUGUCGAAC GAUGCAUGUACGAGUAUUGCCUGAGGG GACAACAGGGUUUCUGUGACCACGCAU GGGAGUUCAAGAAAGAAUGCUACAUAA AGCAUGGAGACACCCUAGAAGUACCAG AUGAAUGCAAAUAG		
GLuc	AUGGGAGUCAAAGUUCUGUUUGCCCUG AUCUGCAUCGCUGUGGCCGAGGCCAAG CCCACCGAGAACAACGAAGACUUCAAC AUCGUGGCCGUGGCCAGCAACUUCGCG ACCACGGAUCUCGAUGCUGACCGCGGG AAGUUGCCCGGCAAGAAGCUGCCGCUG GAGGUGCUCAAAGAGAUGGAAGCCAAU GCCCGGAAAGCUGGCUGCACCAGGGGC UGUCUGAUCUGCCUGUCCCACAUCAAG UGCACGCCCAAGAUGAAGAAGUUCAUC CCAGGACGCUGCCACACCUACGAAGGC GACAAAGAGUCCGCACAGGGCGGCAUA GGCGAGGCGAUCGUCGACAUUCCUGAG AUUCCUGGGUUCAAGGACUUGGAGCCC	MS2-GLuc	Cloned from Addgene plasmid #72888, a gift from Ute Hochgeschwender[10]

	AUGGAGCAGUUCAUCGCACAGGUCGAU CUGUGUGUGGACUGCACAACUGGCUGC CUCAAAGGGCUUGCCAACGUGCAGUGU UCUGACCUGCUCAAGAAGUGGCUGCCG CAACGCUGUGCGACCUUUGCCAGCAAG AUCCAGGGCCAGGUGGACAAGAUCAAG GGGGCCGGUGGUGACUAG		
FLuc	AUGGAAGAUGCCAAAAACAUUAAGAAG GGCCCAGCGCCAUUCUACCCACUCGAA GACGGGACCGCCGGCGAGCAGCUGCAC AAAGCCAUGAAGCGCUACGCCCUGGUG CCCGGCACCAUCGCCUUUACCGACGCAC AUAUCGAGGUGGACAUUACCUACGCCG AGUACUUCGAGAUGAGCGUUCGGCUGG CAGAAGCUAUGAAGCGCUAUGGGCUGA AUACAAACCAUCGGAUCGUGGUGUGCA GCGAGAAUAGCUUGCAGUUCUUCAUGC CCGUGUUGGGUGCCCUGUUCAUCGGUG UGGCUGUGGCCCCAGCUAACGACAUCU ACAACGAGCGCGAGCUGCUGAACAGCA UGGGCAUCAGCCAGCCCACCGUCGUAU UCGUGAGCAAGAAAGGGCUGCAAAAGA UCCUCAACGUGCAAAAGAAGCUACCGA UCAUACAAAAGAUCAUCAUCAUGGAUA GCAAGACCGACUACCAGGGCUUCCAAA GCAUGUACACCUUCGUGACUUCCCAUU UGCCACCCGGCUUCAACGAGUACGACU UCGUGCCCGAGAGCUUCGACCGGGACA AAACCAUCGCCCUGAUCAUGAACAGUA GUGGCAGUACCGGAUUGCCCAAGGGCG UAGCCCUACCGCACCGCACCGCUUGUG UCCGAUUCAGUCAUGCCCGCGACCCCA UCUUCGGCAACCAGAUCAUCCCCGACA CCGCUAUCCUCAGCGUGGUGCCAUUUC ACCACGGCUUCGGCAUGUUCACCACGC UGGGCUACUUGAUCUGCGGCUUUCGGG UCGUGCUCAUGUACCGCUUCGAGGAGG AGCUAUUCUUGCGCAGCUUGCAAGACU AUAAGAUUCAAUCUGCCCUGCUGGUGC CCACACUAUUUAGCUUCUUCGCUAAGA GCACUCUCAUCGACAAGUACGACCUAA GCAACUUGCACGAGAUCGCCAGCGGCG GGGCGCCGCUCAGCAAGGAGGUAGGUG AGGCCGUGGCCAAACGCUUCCACCUAC CAGGCAUCCGCCAGGGCUACGGCCUGA CAGAAACAACCAGCGCCAUUCUGAUCA CCCCCGAAGGGGACGACAAGCCUGGCG CAGUAGGCAAGGUGGUGCCCUUCUUCG AGGCUAAGGUGGUGGACUUGGACACCG GUAAGACACUGGGUGUGAACCAGCGCG GCGAGCUGUGCGUCCGUGGCCCCAUGA UCAUGAGCGGCUACGUUAACAACCCCG AGGCUACAAACGCUCUCAUCGACAAGG ACGGCUGGCUGCACAGCGGCGACAUCG CCUACUGGGACGAGGACGAGCACUUCU UCAUCGUGGACCGGCUGAAGAGCCUGA UCAAAUACAAGGGCUACCAGGUAGCCC CAGCCGAACUGGAGAGCAUCCUGCUGC AACACCCCAACAUCUUCGACGCCGGGG	Ctrl mRNA	Cloned from Addgene plasmid #66812, a gift from Ron Weiss[11]

	UCGCCGGCCUGCCCGACGACGAUGCCG GCGAGCUGCCCGCCGCAGUCGUCGUGC UGGAACACGGUAAAACCAUGACCGAGA AGGAGAUCGUGGACUAUGUGGCCAGCC AGGUUACAACCGCCAAGAAGCUGCGCG GUGGUGUUGUGUUCGUGGACGAGGUGC CUAAAGGACUGACCGGCAAGUUGGACG CCCGCAAGAUCCGCGAGAUUCUCAUUA AGGCCAAGAAGGGCGGCAAGAUCGCCG UGUAA		
4×MTS-EGFP	AUGUCCGUCCUGACGCCGCUGCUGCUG CGGGGCUUGACAGGCUCGGCCCGGCGG CUCCCAGUGCCGCGCGCCAAGAUCCAU UCGUUGGGGGAUCCCUCCGUCCUGACG CCGCUGCUGCUGCGGGGCUUGACAGGC UCGGCCCGGCGGCUCCCAGUGCCGCGC GCCAAGAUCCAUUCGUUGGGGAAGCUU GCCACCUCCGUCCUGACGCCGCUGCUGC UGCGGGGCUUGACAGGCUCGGCCCGGC GGCUCCCAGUGCCGCGCGCCAAGAUCC AUUCGUUGGGGGAUCCCUCCGUCCUGA CGCCGCUGCUGCUGCGGGGCUUGACAG GCUCGGCCCGGCGGCUCCCAGUGCCGC GCGCCAAGAUCCAUUCGUUGGCGGCCG CCGGCUCCGGAGGAAUGACUAGUGUGA GCAAGGGCGAGGAGCUGUUCACCGGGG UGGUGCCCAUCCUGGUCGAGCUGGACG GCGACGUAAACGGCCACAAGUUCAGCG UGUCCGGCGAGGGCGAGGGCGAUGCCA CCUACGGCAAGCUGACCCUGAAGUUCA UCUGCACCACCGGCAAGCUGCCCGUGC CCUGGCCCACCCUCGUGACCACCCUGAC CUACGGCGUGCAGUGCUUCAGCCGCUA CCCCGACCACAUGAAGCAGCACGACUU CUUCAAGUCCGCCAUGCCCGAAGGCUA CGUCCAGGAGCGCACCAUCUUCUUCAA GGACGACGGCAACUACAAGACCCGCGC CGAGGUGAAGUUCGAGGGCGACACCCU GGUGAACCGCAUCGAGCUGAAGGGCAU CGACUUCAAGGAGGACGGCAACAUCCU GGGGCACAAGCUGGAGUACAACUACAA CAGCCACAACGUCUAUAUCAUGGCCGA CAAGCAGAAGAACGGCAUCAAGGUGAA CUUCAAGAUCCGCCACAACAUCGAGGA CGGCAGCGUGCAGCUCGCCGACCACUA CCAGCAGAACACCCCCAUCGGCGACGG CCCCGUGCUGCUGCCCGACAACCACUAC CUGAGCACCCAGUCCGCCCUGAGCAAA GACCCCAACGAGAAGCGCGAUCACAUG GUCCUGCUGGAGUUCGUGACCGCCGCC GGGAUCACUCUCGGCAUGGACGAGCUG UACAAGUAA	MS2-EGFP-MT	Direct fusion of the 4×MTS mitochondria tag and EGFP. 4×MTS was synthesized by GenScript with sequence from GenBank (Access No. DO479429.1)[12]
4×MTS-miRFP670	AUGUCCGUCCUGACGCCGCUGCUGCUG CGGGGCUUGACAGGCUCGGCCCGGCGG CUCCCAGUGCCGCGCGCCAAGAUCCAU UCGUUGGGGGAUCCCUCCGUCCUGACG CCGCUGCUGCUGCGGGGCUUGACAGGC UCGGCCCGGCGGCUCCCAGUGCCGCGC	MS2-iRFP-MT	Direct fusion of 4×MTS mitochondria tag and EGFP. 4×MTS was synthesized by GenScript with

	GCCAAGAUCCAUUCGUUGGGGAAGCUU GCCACCUCCGUCCUGACGCCGCUGCUGC UGCGGGGCUUGACAGGCUCGGCCCGGC GGCUCCCAGUGCCGCGCGCCAAGAUCC AUUCGUUGGGGGAUCCCUCCGUCCUGA CGCCGCUGCUGCUGCGGGGCUUGACAG GCUCGGCCCGGCGGCUCCCAGUGCCGC GCGCCAAGAUCCAUUCGUUGGCGGCCG CCGGCUCCGGAGGAAUGACUAGUGUAG CAGGUCAUGCCUCUGGCAGCCCCGCAU UCGGGACCGCCUCUCAUUCGAAUUGCG AACAUGAAGAGAUCCACCUCGCCGGCU CGAUCCAGCCGCAUGGCGCGCUUCUGG UCGUCAGCGAACAUGAUCAUCGCGUCA UCCAGGCCAGCGCCAACGCCGCGGAAU UUCUGAAUCUCGGAAGCGUACUCGGCG UUCCGCUCGCCGAGAUCGACGGCGAUC UGUUGAUCAAGAUCCUGCCGCAUCUCG AUCCCACCGCCGAAGGCAUGCCGGUCG CGGUGCGCUGCCGGAUCGGCAAUCCCU CUACGGAGUACUGCGGUCUGAUGCAUC GGCCUCCGGAAGGCGGGCUGAUCAUCG AACUCGAACGUGCCGGCCCGUCGAUCG AUCUGUCAGGCACGCUGGCGCCGGCGC UGGAGCGGAUCCGCACGGCGGGUUCAC UGCGCGCGCUGUGCGAUGACACCGUGC UGCUGUUUCAGCAGUGCACCGGCUACG ACCGGGUGAUGGUGUAUCGUUUCGAUG AGCAAGGCCACGGCCUGGUAUUCUCCG AGUGCCAUGUGCCUGGGCUCGAAUCCU AUUUCGGCAACCGCUAUCCGUCGUCGA CUGUCCCGCAGAUGGCGCGGCAGCUGU ACGUGCGGCAGCGCGUCCGCGUGCUGG UCGACGUCACCUAUCAGCCGGUGCCGC UGGAGCCGCGGCUGUCGCCGCUGACCG GGCGCGAUCUCGACAUGUCGGGCUGCU UCCUGCGCUCGAUGUCGCCGUGCCAUC UGCAGUUCCUGAAGGACAUGGGCGUGC GCGCCACCCUGGCGGUGUCGCUGGUGG UCGGCGGCAAGCUGUGGGGCCUGGUUG UCUGUCACCAUUAUCUGCCGCGCUUCA UCCGUUUCGAGCUGCGGGCGAUCUGCA AACGGCUCGCCGAAAGGAUCGCGACGC GGAUCACCGCGCUUGAGAGCUAA		sequence from GenBank (Access No. DO479429.1)[12] miRFP670 sequence was cloned from Addgene plasmid # 136560, a gift from Vladislav Verkhusha[13]
BFP-3×NLS	AUGGUGAGCAAGGGCGAGGAGCUGUUC ACCGGGGUGGUGCCCAUCCUGGUCGAG CUGGACGGCGACGUAAACGGCCACAAG UUCAGCGUGAGGGGCGAGGGCGAGGGC GAUGCCACCAACGGCAAGCUGACCCUG AAGUUCAUCUGCACCACCGGCAAGCUG CCCGUGCCCUGGCCCACCCUCGUGACCA CCCUGAGCCACGGCGUGCAGUGCUUCG CCCGCUACCCCGACCACAUGAAGCAGC ACGACUUCUUCAAGUCCGCCAUGCCCG AAGGCUACGUCCAGGAGCGCACCAUCU UCUUCAAGGACGACGGCACCUACAAGA CCCGCGCCGAGGUGAAGUUCGAGGGCG ACACCCUGGUGAACCGCAUCGAGCUGA AGGGCGUCGACUUCAAGGAGGACGGCA	MS2-BFP-NL	Direct fusion of BFP and 3×NLS nucleus tag. BFP sequence was cloned from Addgene plasmid #55248, a gift from Michael Davidson. 3×NLS was cloned from Addgene plasmid #55110, a gift from Michael Davidson

	ACAUCCUGGGGCACAAGCUGGAGUACA ACUUCAACAGCCACAACAUCUAUAUCA UGGCCGUCAAGCAGAAGAACGGCAUCA AGGUGAACUUCAAGAUCCGCCACAACG UGGAGGACGGCAGCGUGCAGCUCGCCG ACCACUACCAGCAGAACACCCCCAUCG GCGACGGCCCCGUGCUGCUGCCCGACA GCCACUACCUGAGCACCCAGUCCGUGC UGAGCAAAGACCCCAACGAGAAGCGCG AUCACAUGGUCCUGCUGGAGUUCCGCA CCGCCGCCGGGAUCACUCUCGGCAUGG ACGAGCUGUACAAGUCCGGACUCAGAU CUCGAGCUGAUCCAAAAAAGAAGAGAA AGGUAGAUCCAAAAAAGAAGAGAAAGG UAGAUCCAAAAAAGAAGAGAAAGGUAG GAUCCACCGGAUCUAGAUAA		
mCherry -3×NLS	AUGGUGAGCAAGGGCGAGGAGGAUAAC AUGGCCAUCAUCAAGGAGUUCAUGCGC UUCAAGGUGCACAUGGAGGGCUCCGUG AACGGCCACGAGUUCGAGAUCGAGGGC GAGGGCGAGGGCCGCCCCUACGAGGGC ACCCAGACCGCCAAGCUGAAGGUGACC AAGGGUGGCCCCCUGCCCUUCGCCUGG GACAUCCUGUCCCCUCAGUUCAUGUAC GGCUCCAAGGCCUACGUGAAGCACCCC GCCGACAUCCCCGACUACUUGAAGCUG UCCUUCCCCGAGGGCUUCAAGUGGGAG CGCGUGAUGAACUUCGAGGACGGCGGC GUGGUGACCGUGACCCAGGACUCCUCC CUGCAGGACGGCGAGUUCAUCUACAAG GUGAAGCUGCGCGGCACCAACUUCCCC UCCGACGGCCCCGUAAUGCAGAAGAAG ACCAUGGGCUGGGAGGCCUCCUCCGAG CGGAUGUACCCCGAGGACGGCGCCCUG AAGGGCGAGAUCAAGCAGAGGCUGAAG CUGAAGGACGGCGGCCACUACGACGCU GAGGUCAAGACCACCUACAAGGCCAAG AAGCCCGUGCAGCUGCCCGGCGCCUAC AACGUCAACAUCAAGUUGGACAUCACC UCCCACAACGAGGACUACACCAUCGUG GAACAGUACGAACGCGCCGAGGGCCGC CACUCCACCGGCGGCAUGGACGAGCUG UACAAGUCCGGACUCAGAUCUCGAGCU GAUCCAAAAAAGAAGAGAAAGGUAGAU CCAAAAAAGAAGAGAAAGGUAGAUCCA AAAAAGAAGAGAAAGGUAGGAUCCACC GGAUCUAGAUAA	MS2-mCherry-NL	Cloned from Addgene plasmid #55110 mCherry-Nucleus-7, a gift from Michael Davidson

Table S4. Transfection Tables

Transfection experiment	Reporter mRNA(s)	Regulator mRNA (ARCA-capped)	miRNA mimic or protein input mRNA	Transfection control mRNA (ARCA-capped)	Culture condition
Figure 1A	Acap-MS2-EGFP (100 ng)	MCP-VPg (0.5 ng, 0.67 ng, 1 ng, 2 ng, 3.3 ng, 5 ng, 10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure 1B	ARCA-MS2-EGFP (100 ng)	MCP-VPg (0.5 ng, 0.67 ng, 1 ng, 2 ng, 3.3 ng, 5 ng, 10, 13.3 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure 1C, S1E	ARCA-MS2-EGFP (100 ng)	MCP-VPg (13.3 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
	m7G-MS2-EGFP (100 ng)				
	3Met-MS2-EGFP (100 ng)				
	Acap-MS2-EGFP (100 ng)				
Figure 1D, S2A, B	Acap-MS2-EGFP (50 ng) and ARCA-MS2-mCherry (50 ng)	MCP-VPg (0.6 ng, 1.3 ng, 2.5 ng, 5 ng, 10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure 2A, S4A	Acap-MS2-EGFP (200 ng)	miR-21-MCP-VPg (5 copies, 4 ng)	miR-21 mimic (8 pmol)	iRFP670 (25 ng)	Confocal dish/2 mL
	ARCA-MS2-EGFP (200 ng)	miR-21-MCP-VPg (5 copies, 80 ng)			
Figure 2B, S5A	anti-miR-21-EGFP (5 copies 100 ng)	N/A	miR-21 mimic (1 pmol)	iRFP670 (15 ng)	48-well plate/0.25 mL
	Acap-MS2-EGFP (100 ng)	miR-21-MCP-VPg (5 copies, 2 ng)			
Figure 2C, S5A	2×MS2-EGFP (100 ng)	miR-21-MCP (5 copies, 100 ng)	miR-21 mimic (1 pmol)	iRFP670 (15 ng)	48-well plate/0.25 mL
	ARCA-MS2-EGFP (100 ng)	miR-21-MCP-VPg (5 copies, 10 ng)			
Figure 2D, S5C, D	Acap-MS2-EGFP (50 ng) and ARCA-MS2-BFP (50 ng)	miR-21-MCP-VPg (5 copies, 10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure 3A	Acap-MS2-CLuc (15 ng) and ARCA-	MCP-VPg (10 ng)	N/A	N/A	48-well plate/0.25 mL

	MS2-GLuc (5 ng)				
Figure 3B	Acap-PP7-EGFP (50 ng) and ARCA-PP7-mCherry (50 ng)	PCP-VPg (100 ng)	N/A		48-well plate/0.25 mL
Figure 3D	Acap-PP7-EGFP (50 ng)	2×MS2-PCP-VPg (N1ψ modified, 2 ng)	Ctrl mRNA or MCP (N1ψ modified, 50 ng)	iRFP670 (15 ng)	48-well plate/0.25 mL
	ARCA-PP7-EGFP RNA (50 ng)	2×MS2-PCP-VPg (N1ψ modified, 10 ng)			
Figure 3E, S6B	MS2-EGFP-MT (25 ng), MS2-miRFP670-MT (25 ng), MS2-BFP-NL (25 ng), MS2-mCherry-NL (25 ng)	MCP-VPg (80 ng)	N/A	N/A	Confocal dish/2 mL
Figure S1A	Acap-MS2-EGFP (100 ng)	MCP-VPg (10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S1B	ARCA-MS2-EGFP (100 ng)	MCP-VPg (10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S1C	Acap-MS2-EGFP (100 ng)	MCP (6.25 ng), VPg (6.5 ng), MCP-VPg (10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S1D	ARCA-MS2-EGFP (100 ng)	MCP (6.25 ng), VPg (6.5 ng), MCP-VPg (10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S2C	Acap-MS2-EGFP (50 ng) and ARCA-MS2-mCherry (50 ng)	MCP-VPg (10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S3A	Acap-MS2-EGFP (100 ng)	miR-21-MCP-VPg (4 copies with no nucleotide modification, N1ψ modified, 5mC/ψ modified, 2 ng)	miR-21 mimic (1 pmol)	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S3B	ARCA-MS2-EGFP (100 ng)	miR-21-MCP-VPg (4 copies with no nucleotide modification, N1ψ modified,	miR-21 mimic (1 pmol)	iRFP670 (15 ng)	48-well plate/0.25 mL

		5mC/ψ modified, 10 ng)			
Figure S3C	Acap-MS2-EGFP (100 ng)	miR-21-MCP-VPg (5 copies, 2 ng), same molar amounts of miR-21-MCP-VPg (1, 4, 8 copies)	miR-21 mimic (1 pmol)	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S3D	ARCA-MS2-EGFP (100 ng)	miR-21-MCP-VPg (5 copies, 2 ng), same molar amounts of miR-21-MCP-VPg (1, 4, 8 copies)	miR-21 mimic (1 pmol)	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S4B	Acap-MS2-EGFP (50 ng) and ARCA-MS2-BFP (50 ng)	miR-302-MCP-VPg (10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S5B, C, D	Acap-MS2-EGFP (50 ng) and ARCA-MS2-BFP (50 ng)	MCP-VPg (10 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
Figure S6A	Acap-PP7-EGFP (100 ng)	PCP-VPg (100 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL
	ARCA-PP7-EGFP (100 ng)	PCP-VPg (100 ng)	N/A	iRFP670 (15 ng)	48-well plate/0.25 mL

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