Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information (SI)

A microRNA-gated thgRNA platform for multiplexed activation of gene expression in mammalian cells

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Methods

Plasmid construction for GFP activation studies:

For the plasmids used in HeLa studies, pcDNA™3.1(+) vector was used as the backbone for most constructs, purchased from Invitrogen™ through ThermoFisher. For activation studies, *Sp* dCas9 VPR was PCR amplified from plasmid, *Sp*-dCas9-VPR, which was a gift from George Church (Addgene plasmid #63798; http://n2t.net/addgene:63798 ; RRID: Addgene_63798) (1). Using restriction enzymes, *Sp*-dCas9-VPR was inserted into pcDNA™3.1(+) to make CRISPRa VPR. For the reporter used in activation studies, the minimal CMV promoter was adapted from (2) to drive expression of GFP. Two tandem repeats of the gRNA target site were inserted upstream of the minimal CMV promoter. For expression of gRNA driven by hU6 promoter, hU6 promoter *Sp* sgRNA scaffold was PCR amplified from plasmid, pLV-hU6-sgRNA hUbC-dCas9-KRAB-T2a-Puro, which was a gift from Charles Gersbach (Addgene plasmid #71236; http://n2t.net/addgene:71236 ; RRID: Addgene_71236) (3). All gRNAs were inserted into vector using BsmBI restriction enzyme. sgRNA, thgRNA and trigger HH thgRNA oligos were ordered from a commercial vendor (Integrated DNA Technologies®, Inc., Coralville, IA, USA), treated with PNK (T4 Polynucleotide Kinase) (New England BioLabs®, Inc.), annealed, and ligated. In this approach, both gRNA and dCas9-VPR are expressed from the same plasmid. For microRNA-inducible constructs expression of gRNA, all gRNAs had flanking miRx (either miR21 or miR294) binding sites PCR amplified on both the 5' and 3'ends of gRNAs. These cassettes were inserted using restriction enzymes into pcDNA™3.1(+). For synthetic trigger constructs, oligos with the hammerhead (HH) ribozyme, 5' hairpin (to improve stability), trigger and hepatitis delta virus (HDV) ribozyme were ordered from IDT, PNK treated and annealed. The synthetic trigger cassette was inserted downstream of miRx-thgRNA-miRx so that both the thgRNA and synthetic trigger were expressed from same CMV promoter. For studies with full-length *mCherry* or *BFP* used to activate hU6 driven thgRNA, *mCherry* or *BFP* was inserted into the multiple cloning site of pcDNA™3.1(+) and delivered as an additional plasmid. For studies with *mCherry* expressed to activate miRx-inducible mCh thgRNA, *mCherry* was inserted using

restriction enzymes downstream of the SV40 promoter in pcDNA™3.1(+), in the same plasmid that expressed the miRx-thgRNA-miRx, to consolidate the number of plasmids delivered.

Plasmid construction for protein degradation studies:

Depending on single or dual input protein degradation, either miRx-gRNA-miRx or miRx-gRNA-miRx HH trig * HDV plasmids were used to express gRNA, respectively. CRISPRa VPR, described above, was used for these studies as well to express dCas9-VPR. For the reporter in protein degradation studies, CMV driven GFP was used in the studies when GS2-IpaH9.8 was activated. For studies with Nsa5-IpaH9.8, the SH2 domain of the SHP2 protein was fused to GFP and expressed from CMV promoter. The SH2 domain was PCR amplified from pJ3 SHP2 WT, which was a gift from Ben Neel (4) and inserted in frame upstream of GFP. For the protein degradation component, the minimal CMV promoter was adapted from (5) to drive expression of the protein degradation complex when activated by the conditional gRNA. The protein degradation component consisted of a designer binding protein (DBP) fused to IpaH9.8 or the mutated IpaH9.8 (C337A). The DBP was either GS2, which has an affinity for GFP or Nsa5, which has an affinity for the SH2 domain of the SHP2 protein. Two tandem repeats of the gRNA target site were inserted upstream of the minimal CMV promoter. We received plasmid containing GS2-IpaH9.8 as a gift from Matt DeLisa (6). The catalytically active GS2-IpaH9.8 was PCR amplified and inserted downstream of the minimal CMV promoter, replacing GFP for protein degradation studies. For the studies with the other DBP, Nsa5, the sequence for Nsa5 was ordered as a gBlock fragment from IDT, PCR amplified and inserted upstream of IpaH9.8 (7).

Cell Culture:

HeLa cells were cultivated as per directed by ATCC. HeLa cells were maintained in complete growth media containing MEM, 1X, 10% FBS (fetal bovine serum), 1% penicillin-streptomycin.

Cells were passaged every third or fourth day depending on confluency. For subculturing, aspirate and discard the wasted culture media. The cell layer was washed with PBS (phosphate-buffered saline) (1X, pH 7.4) and the used PBS aspirated. 1 mL of Trypsin-EDTA (Corning® Trypsin-EDTA 1X, 0.05% Trypsin/0.53 mM EDTA) (Corning®, Inc., Corning, NY, USA) was added, to sufficiently cover the cell layer, and incubated for 5 min at 37℃. When the cells appear to be detached, 8-10 mL of complete growth media was added to inactive trypsin. Appropriate aliquots of the cell suspension were transferred to new culture vessels, generally T-75 flasks (CellTreat® Scientific Products, Pepperell, MA, USA), and fresh complete growth media was added to bring volume up to 12 mL. HEK293T cells were cultured as suggested by ATCC. Cell culture numbers for seeding densities and appropriate volumes were used as described by Thermo Fisher Scientific Cell Culture Protocols. For most transfection experiments, HeLa cells were seeded in 12-well plates (CellTreat® Scientific Products Pepperell, MA, USA). HeLa cells were seeded at $1x10^5$ cells/mL in 1 mL of complete growth media (MEM, 1x, 10%FBS, 1% Penicillin/Streptomycin). After 24 hours generally, cells were transfected around 80-90% confluency.

GFP activation studies:

For most transfection experiments, HeLa or HEK293T cells were seeded in 12-well plates (CellTreat® Scientific Products Pepperell, MA, USA). All experiments were performed in HeLa except experiment noted in Fig. S6, which were performed in HEK293T. Cells were seeded at $1x10^5$ cells/mL in 1 mL of complete growth media (MEM, 1x, 10%FBS, 1% Penicillin/Streptomycin). After 24 hours generally, cells were transfected around 80-90% confluency. All transfections were carried out using Lipofectamine™ 3000 (ThermoFisher) using the manufacturer's recommended protocol. There were three plasmids delivered simultaneously for all the microRNA-inducible studies, reporter: CRISPRa VPR: miRx-gRNA-miRx. The mass ratio for most of the studies was 10 ng reporter: 300 ng CRISPRa VPR: 300 ng miRx-gRNA-miRx. For the studies done with full-length *mCherry*, the amount of reporter

delivered was increased to 100 ng. Between 8-18 hrs post-transfection, cells were aspirated, washed with PBS, and fresh growth media was added. Experiments with miRx-thgRNA-miRx directed GFP activation were generally analyzed at 24 hrs. post transfection. For protein degradation studies using GS2-IpaH9.8 where miR-inducible gRNA was used, we delivered 1:5:15:30 molar ratio of GFP: protein degradation: CRISPRa VPR: miRx-gRNA-miRx (10 ng: 50 ng: 300 ng: 300 ng). For each sample the total DNA delivered was kept constant. For protein degradation studies using Nsa5-IpaH9.8 where miR-inducible gRNA was used, we delivered 100 ng: 50 ng: 300 ng: 300 ng of SH2-GFP: protein degradation: CRISPRa VPR: miRx-gRNA-miRx. Between 8-18 hrs post-transfection, cells were aspirated, washed with PBS, and fresh growth media was added. Cells were generally analyzed 48 hrs post-transfection, unless otherwise noted.

Flow cytometry:

For flow cytometry experiments, HeLa cells were generally transfected in 12-well plates (CellTreat® Scientific Products). HeLa cells were washed with 1 mL/well PBS (phosphate-buffered saline) (1X, pH 7.4), typically 1mL PBS/well and then trypsinized with 100-200 μL of trypsin-EDTA (Corning® Trypsin-EDTA 1X, 0.05% Trypsin/0.53 mM EDTA) for 5 minutes, incubated at 37℃. Once cells appeared detached, add 1 mL of complete growth media to inactivate trypsin. Cells were centrifuged for 5-10 minutes at 4°C at 800 x g. The media was aspirated, and cells were resolubilized in cold PBS. Solution was transferred to flow cytometry tube. The flow cytometer used for all experiments is the ACEA NovoCyte® Flow Cytometer (Agilent, Santa Clara, CA, USA). The flow cytometer was set up, maintained, and run as instructed from manufacturer's recommendation. The cells were gated for live cells and then gated to exclude any doublets. 15,000 gated events were collected for each sample. All experiments detected levels of GFP or GFP fusion fluorescence using the configuration for FITC (Ex: 488 nm/ Detection: 530 nm). For *mCherry* delivery studies, all experiments detected levels of *mCherry* using the configuration for PE-Cy5 (Ex:488 nm/ Detection: 660 nm). Cells were

additionally gated for *mCherry* expression. The mean fluorescence intensity values are shown as the results.

Statistical analysis:

All experiments were performed in triplicates, with a couple of noted exceptions which were performed with fewer, $n<3$. The results were expressed as means ± standard deviations (SD). Statistical significance was analyzed using the student t-tests. $P < 0.05$ was considered statistically significant throughout the experiments. $P < 0.05$ was represented as *, $P < 0.01$ was represented as **, $P < 0.001$ was represented as *** and no statistically significant difference (NS).

Supplemental Method References:

1. Chavez, A.; Scheiman, J.; Vora, S.; Pruitt, B. W.; Tuttle, M.; P R Iyer, E.; Lin, S.; Kiani, S.; Guzman, C. D.; Wiegand, D. J.et al. Highly efficient Cas9-mediated transcriptional programming. *Nature Methods* **2015,** *12* (4), 326.

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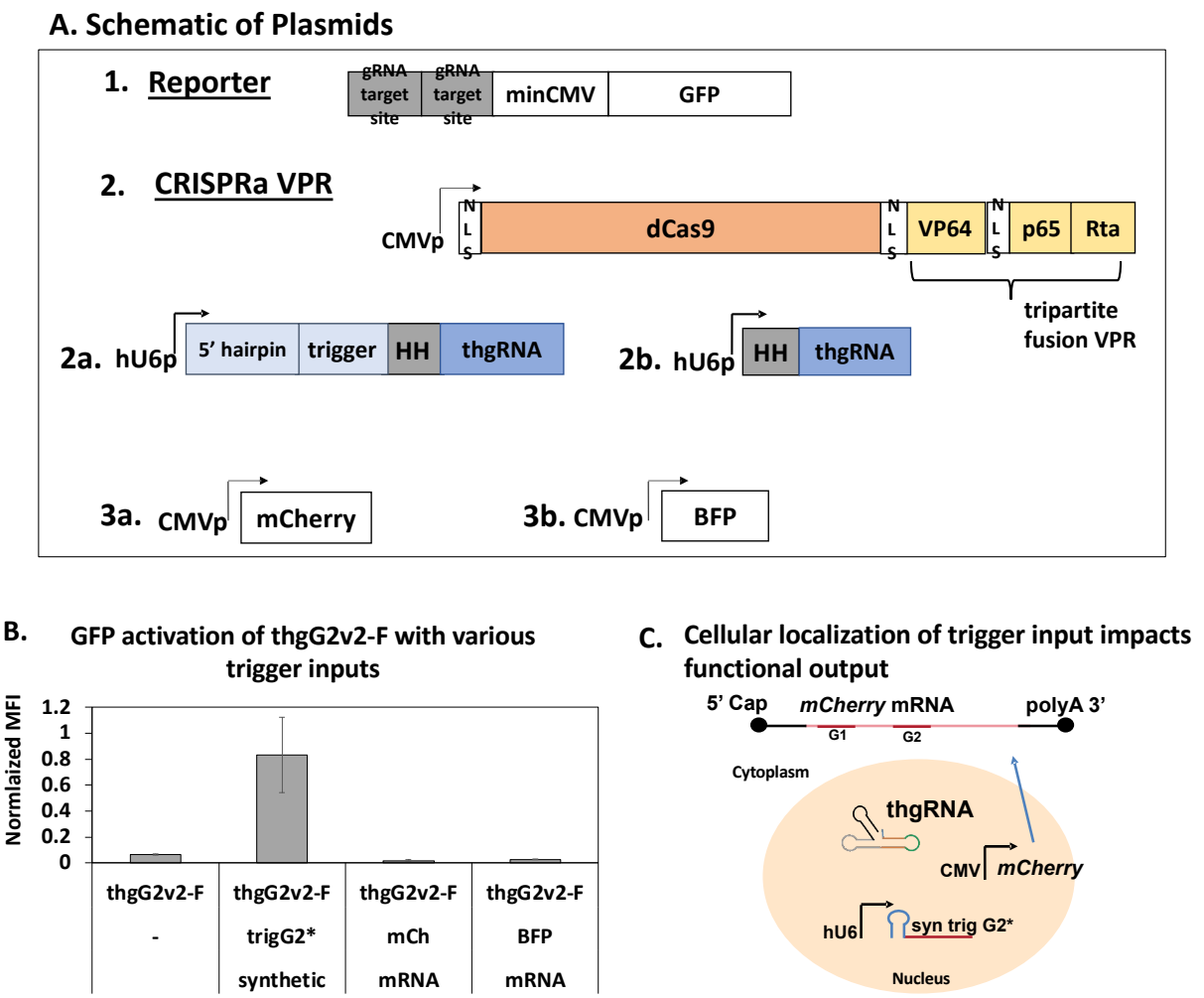

Figure S1. Full-length mCherry mRNA is unable to activate thg2v2-F expressed using a hU6 promoter and localized to the nucleus. (A) Schematic of plasmids used for the *mCherry* studies. Three plasmids were transfected simultaneously. The reporter consisted of two tandem repeats of the CRISPR target site upstream of the minimal CMV promoter, which drives expression of GFP. dCas9-VPR was expressed from the CMV promoter. thgRNA was expressed from a hU6 promoter. NLS = SV40 nuclear localization signal; HH = self-cleaving hammerhead ribozyme. (B) thg2v2thg-F expressed from the hU6 promoter did not activate GFP expression when *mCherry* was co-transfected. In contrast, a synthetic trigger generated from ribozyme cleavage successful activated GFP expression. BFP was co-transfected as a control. (C) Cellular localization of hU6 driven synthetic trigger compared to exogenously expressed *mCherry* mRNA for the trigger input. The export from nucleus of *mCherry* mRNA prevents sufficient residence time for TMSD to occur. Data are the average of three or more biological replicates (separately transfected wells), and the error is the standard deviation (SD) of the mean. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS means no statistically significant difference. MFI is the mean fluorescence intensity.

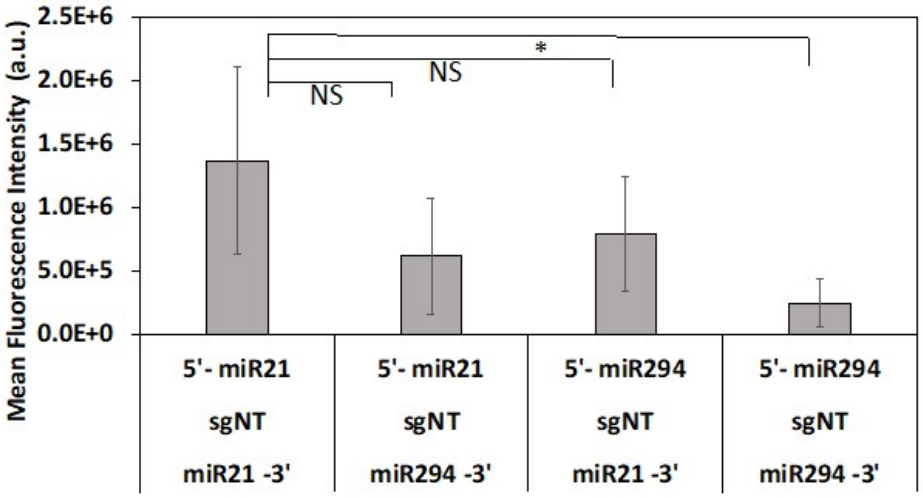

Figure S2. Both 5' cap and 3' polyA tail processing by microRNA are needed for sgRNA activation. While up to 50% activation was detected when either the 5' cap or 3' polyA tail was removed, dual processing was necessary for full function. Similar observations for partial function of Cas9/gRNA gene regulation with either 5' or 3' end modifications or extensions were observed in other studies[8,9]. Data are the average of three or more biological replicates (separately transfected wells), and the error is the standard deviation (SD) of the mean. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS means no statistically significant difference.

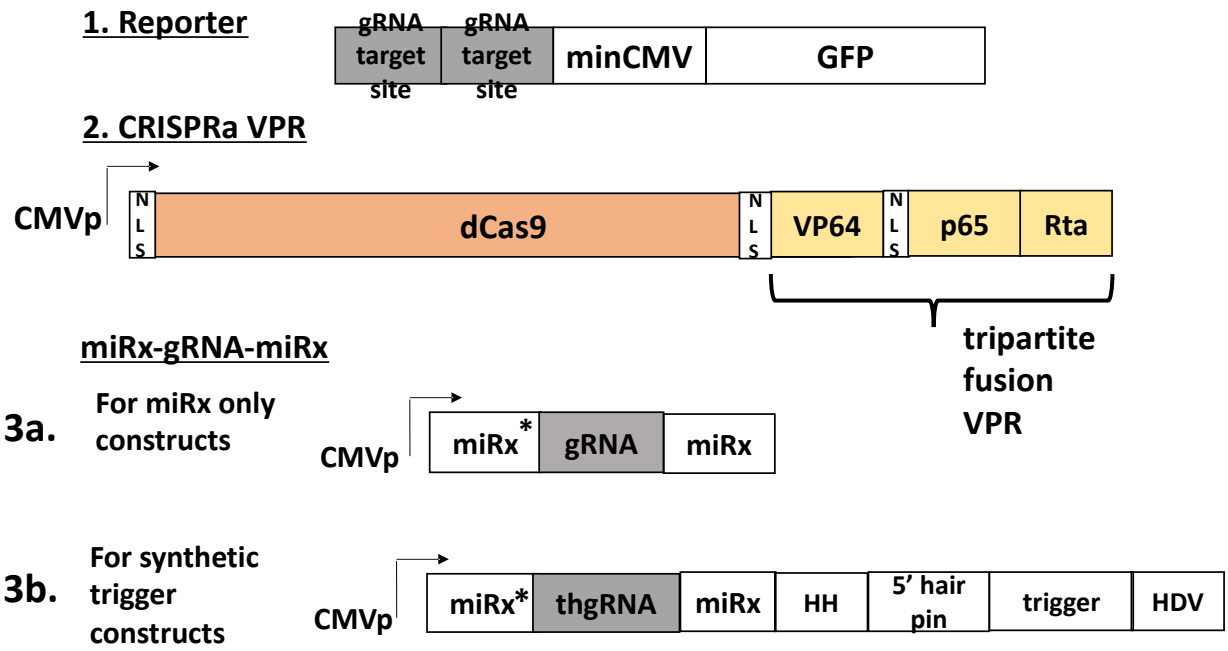

Figure S3. Schematic of plasmids used for the miRx and combined miRx/thgRNA experiments. Three plasmids were transfected simultaneously. The reporter consisted of two gRNA target sites upstream of the minimal CMV promoter driving expression of GFP. dCas9-VPR was expressed from the CMV promoter on a single plasmid. (A) For miRx-gRNA-miRx constructs, the gRNA was expressed from the CMV promoter with miRx binding sites on the 5' and 3' end. (B) For synthetic trigger constructs, downstream of the cassette, miRx-thgRNA-miRx, the synthetic trigger cassette was inserted containing hammerhead (HH) ribozyme, 5'hairpin, trigger, hepatitis delta virus (HDV) ribozyme. Both thgRNA and synthetic trigger were expressed from same CMVp and ribozymes would function to release the synthetic trigger from transcribed RNA. NLS = SV40 nuclear localization signal, HH = self-cleaving ribozyme hammerhead, HDV = self-cleaving ribozyme hepatitis delta virus.

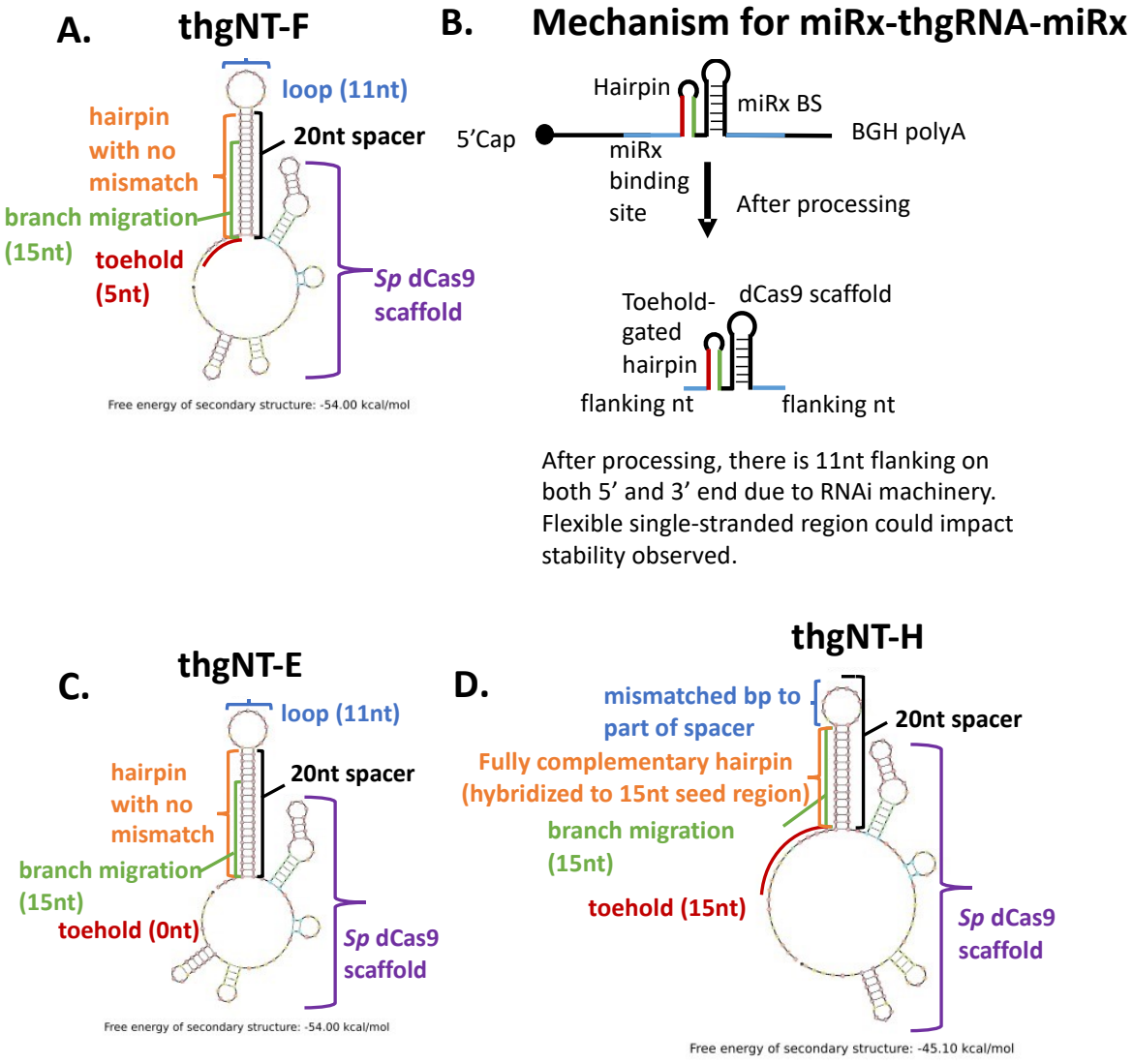

Figure S4. Use of different thgRNA designs for miRNA-gated activation. (A) thgNT-F contains a 5nt toehold region, 15nt loop, and a fully hybridized stem region. (B) When miRx-thgRNA-miRx is expressed from CMV promoter with miRx binding sites on the 5' and 3' and processed, there are 11nt flanking on both sides. (C) thgNT-E contains a 0nt toehold region, 15nt loop, and a fully hybridized stem region. The lack of toehold should prevent toehold-mediated strand displacement from occurring. (D) thgNT-H contains a 15nt toehold, 15nt branch migration region, and no flexible loop.

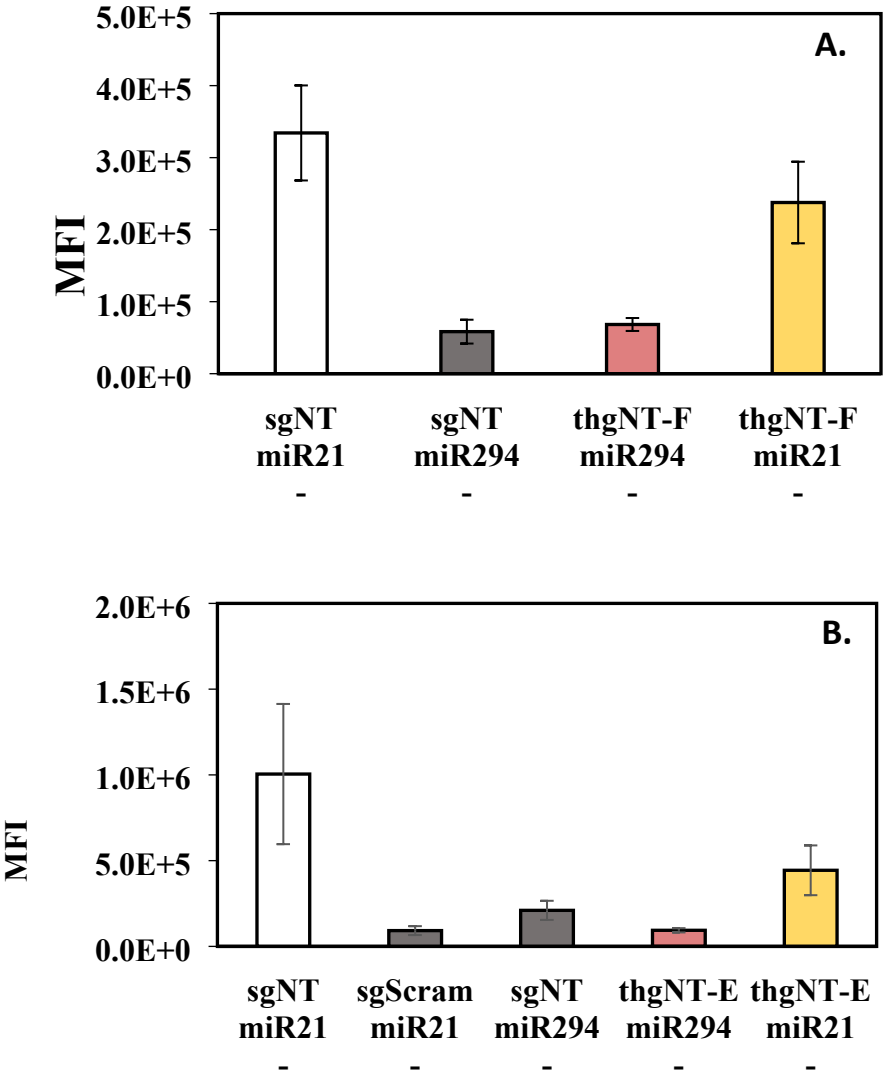

Figure S5. Evaluation of miR-gated thgNT-F and thgNT-E for background leak. (A) For miR-21-gated thgNT-F, we observed a leak in thgNT-F of 39%, while no leak was detected for the miR-294-gated thgNT-F. (B) For thgNT-E without a toehold sequence, a small leak was still detected, indicating the loop region is interacting with the flanking 11 nt left after miRNA-mediated processing. Data are the average of three or more biological replicates (separately transfected wells), and the error is the standard deviation (SD) of the mean. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS means no statistically significant difference. MFI is mean fluorescence intensity.

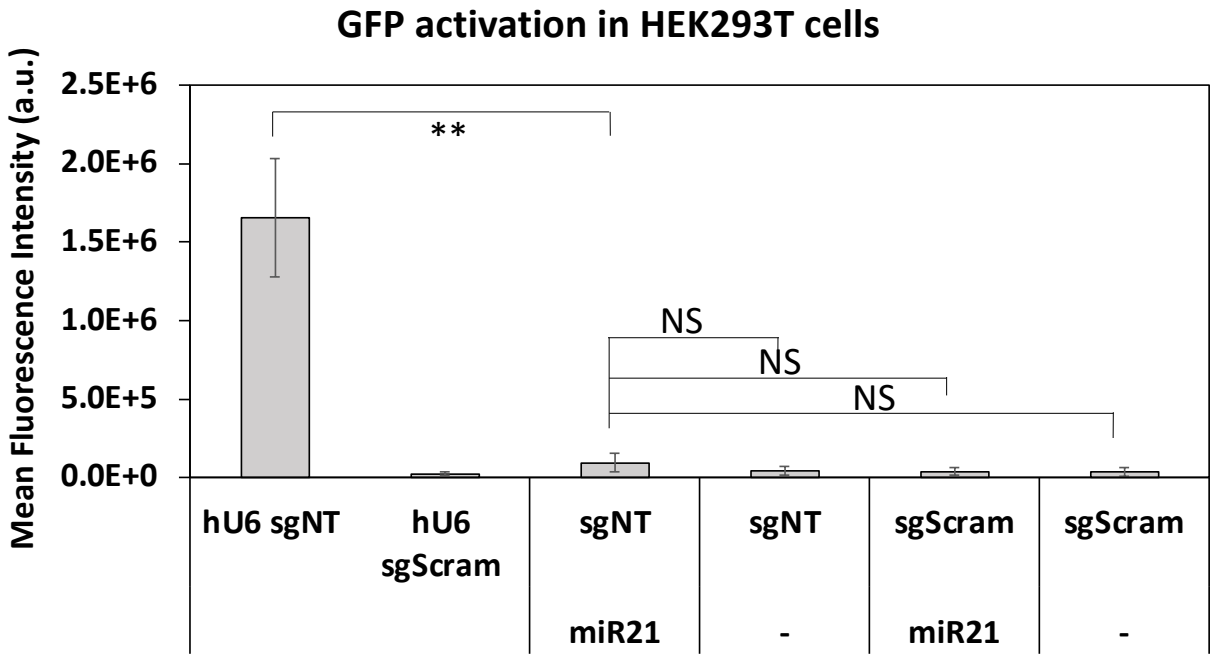

Figure S6. HEK293T cells do not show miR21-activated GFP expression. sgNT and sgScram expressed from hU6 promoter are positive and negative controls for HEK293T. HEK293T express low to negligible levels of miR21, so are essentially miR21-null cells. There is no observable GFP activation with miR21-sgNT-miR21. Data are the average of three or more biological replicates (separately transfected wells), and the error is the standard deviation (SD) of the mean. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS means no statistically significant difference.

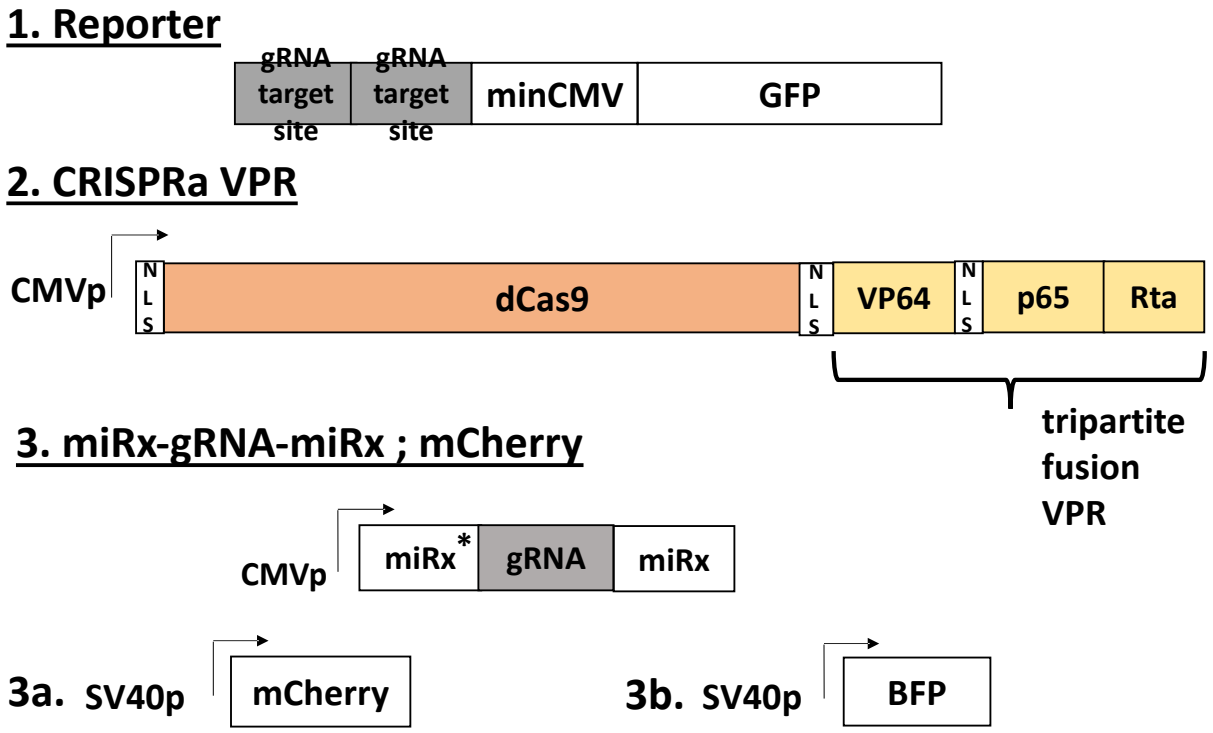

Figure S7. Schematic of plasmids for using full-length mCherry mRNA for activation. Three plasmids were transfected simultaneously. The reporter was the same as the previous experiments. dCas9-VPR was expressed from the CMV promoter. gRNA was expressed from the CMV promoter with miRx binding sites on the 5' and 3' end. Full-length mCherry was inserted downstream of the SV40 promoter on the same plasmid containing the gRNA. A full-length BFP was used as a control. For miRx, in HeLa cells the miR used for targeting was miR21 and miR294 for negative control. NLS = SV40 nuclear localization signal

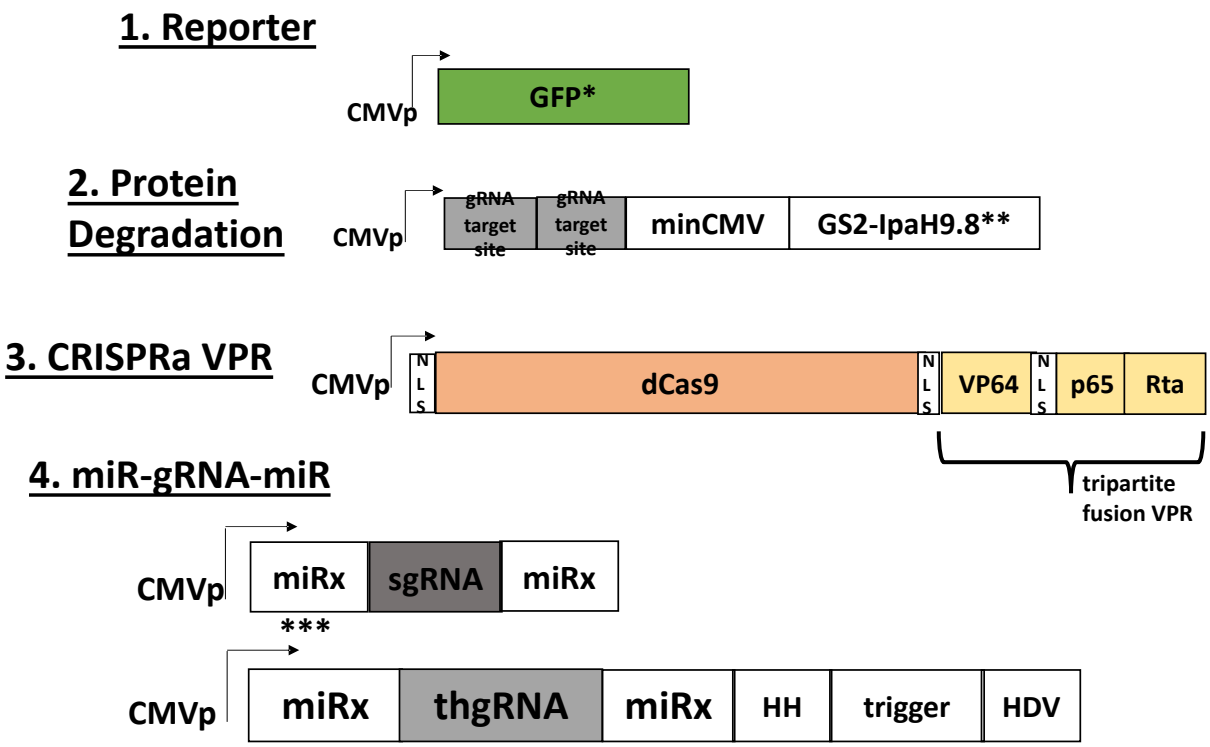

Figure S8. Schematic of plasmids for conditional protein degradation. In this experiment, four plasmids were delivered simultaneously. The reporter protein was expressed using a CMV promoter. The protein degradation plasmid expressed either GS2-IpaH9.8 or Nsa5-IpaH9.8 from a minimal promoter that is activation by dCas9-VPR binding. The dCas9-VPR was expressed by a CMV promoter on a separate plasmid. For the thgRNA/miRx combined plasmids, the miRx binding sites are on the 5' and 3' end of the thgRNA followed by a hammerhead (HH) ribozyme, synthetic trigger, and hepatitis delta virus (HDV) ribozyme. The ribozymes self-cleave and release the synthetic trigger all from a single promoter. NLS = SV40 nuclear localization signal, HH = self-cleaving hammerhead ribozyme, HDV = self-cleaving hepatitis delta virus ribozyme.

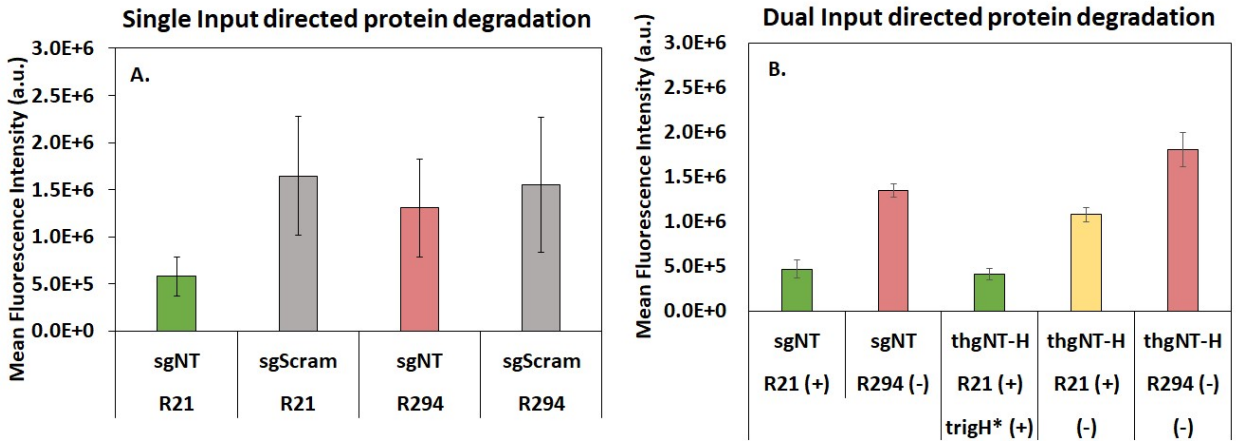

Figure S9. Nsa5-directed conditional protein degradation using either a single- and dual-input design. (A) Using miR21 to induce mature sgNT, we targeted conditional protein degradation of GFP through activation of GS2-IpaH9.8 and observed 2.4-fold knockdown as compared to the positive control, which resulted in 3.7-fold knockdown. (B) When miR21 is used as the input, mature sgNT activated expression of Nsa5-IpaH9.8 and resulted in 2.3-fold knockdown of SH2-GFP. Data is the average of five biological replicates (five separate wells transfected), and the error is the standard deviation (SD) of the mean. *** $p<0.001$, NS $p>0.05$.

gRNA name	Notes on design	Trigger input	Promoter used	Used in experiment
sgNT	Used as sgRNA alone expressed from hU6 or CMV promoter, or with miR binding sites on 5' and 3' end expressed from CMV promoter	N/A	hU6 or CMV; denoted in study	
sgScram	Used as sgRNA alone expressed from hU6 or CMV promoter, or with miR binding sites on 5' and 3' end expressed from CMV promoter	N/A	hU6 or CMV; denoted in study	
g2v2thg-F	5nt toehold 11nt flexible loop	Synthetic G2* trigger OR *mCherry* mRNA	hU6 only	See Fig. S1
miRx-thgNT-F-miRx	5nt toehold 11nt flexible loop	Synthetic trigF*	CMV only	See Fig. S4 & S5, previous study[13] used hU6 thgNT-F
miRx-thgNT-E-miRx	0nt toehold 11nt flexible loop	Synthetic trigE*	CMV only	See Fig. S4 & S5, previous study[13] used hU6 thgNT-E
miRx-thgNT-H-miRx	15nt toehold 0nt flexible loop	Synthetic trigH*	CMV only	See Fig. 2, Fig. 4, Fig. S4 & S9, previous study[13] used hU6 thgNT-H
miRx-g2v2 sg-miRx	Used as controls for *mCherry* experiment	N/A	CMV only	See Fig. 3
miRx-g2v2thg-H-miRx	15nt toehold 0nt flexible loop	Synthetic G2* trigger OR *mCherry* mRNA	CMV only	See Fig. 3

Nomenclature Rules

sg = single guide RNA (spacer + scaffold only)
thg = toehold-gated gRNA design (contains toehold, branch migration, loop, spacer, scaffold)
NT = arbitrary identifier given to spacer sequence used for preliminary designs and system characterization
Scram = scrambled NT sequence
-E,-F,-H = additional versions of thgRNA – all have same NT spacer sequence but toehold length and flexible loop vary
g2v2 = designed for mCherry mRNA trigger input G2 region, version 2 design
miRx = denotes flanking miR binding sites on both 5' and 3' end of gRNA

Supplemental Table 1. Summary of gRNAs used in study

Sequences used in studies with miRx binding sites on 5' and 3' ends							
	5' GGG	**toehold**	**branch migration**	**remaining stem**	**loop**	**spacer**	**scaffold**
thgNT-B	GGG	AGTTTGATTACATTG	CTGCTTACTGGCTTA	TCGAg	atAACACTcgg	TTCGATAAGCCAGTAAGCAG	*
thgNT-E	GGG		CTGCTTACTGGCTTA	TCGAg	atAACACTcgg	TTCGATAAGCCAGTAAGCAG	*
thgNT-F	GGG	CATTG	CTGCTTACTGGCTTA	TCGAg	atAACACTcgg	TTCGATAAGCCAGTAAGCAG	*
thgNT-H	GGG	AGTTTGATTACATTG	CTGCTTACTGGCTTA	catgc		TTCGATAAGCCAGTAAGCAG	*
mCh G2v1 thg-H	GGG	GCCCTCGCCCTCGCC	CTCGATCTCGAACTC	TAGTT		GGTATGAGcagGAGATCGAG	*
mCh G2v2 thg-H	GGG	GCCCTCGCCCTCGCC	CTCGATCTCGAACTC	TAGTT		GGTATGAGttcGAGATCGAG	*
mCh G1v2 thg-H	GGG	TGATGGCCATGTTAT	CCTCCTCGCCCTTGC	aatgt		GGTAAGCAaggGCGAGGAGG	*
sgNT						GTTCGATAAGCCAGTAAGCAG	*
sgScram						AATTCGCACTGTCAGGGTCG	*
sgG1v2						GGTAAGCAaggGCGAGGAGG	*
sgG2v1						GGTATGAGCAGGAGATCGAG	*
sgG2v2						GGTATGAGttcGAGATCGAG	*
microRNA target sites added to 5' and 3' end							
miR21 binding site	5'-tcaacatcagtctgataagcta						
miR294 binding site	5'-acacacaaaagggaagcacttt						

Supplemental Table 2. Sequences of gRNAs used in combination with microRNA-inducible approach.

Primers used to add miRx binding sites to 5' and 3' to gRNA for miR-inducible approach	
VMH 231 NheI miR21 SpT18 FP	atcggctagctcaacatcagtctgataagctaTTCGATAAGCCAGTAAGCAGg
VMH 233 Nhei miR21 SpScram FP	atcggctagctcaacatcagtctgataagctaAATTCGCACTGTCAGGGTCG
VMH 247 NheI miR294 corrected SpT18 FP	atcggctagcacacacaaaagggaagcactttTTCGATAAGCCAGTAAGCAGg
VMH 236 NheI miR294 SpScram FP	atcggctagcacacacaaaagggaagcactttAATTCGCACTGTCAGGGTCG
VMH 237 Nhei SpT18 FP	atcggctagcTTCGATAAGCCAGTAAGCAG
VMH 238 NheI SpScram FP	atcggctagcAATTCGCACTGTCAGGGTCG
VMH 250 NheI miR21 SpthgNT-B FP	atcggctagctcaacatcagtctgataagctagggAGTTTGATTACATTGCTGCTTAC
VMH 249 NheI miR294 SpthgNT-B FP	atcggctagcacacacaaaagggaagcactttgggAGTTTGATTACATTGCTGCTTAC
VMH 275 miR21-thgNT-E(0nt) FP	atcggctagctcaacatcagtctgataagctaGGGCTGCTTACTGGCTTATCG
VMH 276 miR294-thgNT-E(0nt) FP	atcggctagcacacacaaaagggaagcactttGGGCTGCTTACTGGCTTATCG
VMh 412 NheI miR21 thgNT-F FP	atcggctagctcaacatcagtctgataagctagggCATTGCTGCTTACTGG
VMH 413 NheI miR294 thgNT-F FP	atcggctagcacacacaaaagggaagcactttgggCATTGCTGCTTACTGG
VMH 301 NheI miR21-mCh thg G2 FP	atcggctagctcaacatcagtctgataagctagggGCCCTCGCCCTCGCC
VMH 303 NheI miR21-mCh G2v1 sgRNA FP	atcggctagctcaacatcagtctgataagctaGGTATGAGCAGGAGATCGAGG
VMH 304 NheI miR21-mCh G2v2 sgRNA FP	atcggctagctcaacatcagtctgataagctaGGTATGAGTTCGAGATCGAGG
VMH 349 Nhei miR21- mch G1v2 sgRNA FP	atcggctagctcaacatcagtctgataagctaGGTAAGCAAGGGCGAGGAG
VMH 350 Nhei miR21- mch G1v2 thgRNA FP	atcggctagctcaacatcagtctgataagctaGGGTGATGGCCATGTTATCCTC
VH 473 miR294-G2v1sgRNA FP	atcggctagcacacacaaaagggaagcactttGGTATGAGcagGAGATCGAG
VH 474 miR294-G2v2sgRNA FP	atcggctagcacacacaaaagggaagcactttGGTATGAGttcGAGATCGAG
VH 476 miR294-G2v2mChthg FP	atcggctagcacacacaaaagggaagcactttgggGCCCTCGCCCTCGC
VH 477 miR294-G1v2mChthg FP	atcggctagcacacacaaaagggaagcactttgggTGATGGCCATGTTATCCTCCTCG
VH 475 miR294-G1v2sgRNA FP	atcggctagcacacacaaaagggaagcactttGGTAAGCAaggGCGAGGAG
VMH 232 HIndIII miR21 SpScaffold RP	tgacaagctttagcttatcagactgatgttgaGCACCGACTCGGTGC
VMH 239 HindIII Sp scaffold RP	tgacaagcttGCACCGACTCGGTGC
VMH 248 HindIII miR294 corrected SpScaffold RP	tgacaagcttaaagtgcttccctttgtgtgtGCACCGACTCGGTGC
VMH 302 HIndIII miR21- Sp gRNA scaffold RP	tgacaagctttagcttatcagactgatgttgaGCACCGACTCGGTGCCAC
Primers used for protein degradation components	
VMH 269 NheI GS2-IpaH9.8 FP	acgtgctagcATGGTTTCTTCTGTTCCGACCAAAC
VMH 270 XbaI GS2-IpaH9.8 RP	acgttctagaTTAGTGATGATGATGATGATGTCCCTTGTC
VMH 271 HindIII GS2-IpaH9.8 RP	acgtaagcttTTAGTGATGATGATGATGATGTCCCTTGTC
VMH 272 NotI GS2-IpaH9.8 FP	acgtgcggccgcATGGTTTCTTCTGTTCCGACCAAAC
VMH 330 NotI EcoRI IpaH9.8 -for SHP2 FP	actcgcGCGGCCGCGAATTCCTGGCTGATGCCGTG
VMH 331 IpaH9.8- for SHP2 RP	GGGTTTAAACGGGCCCTctag
VMH 332 Nsa5FP	atcgtcGCGGCCGCgccaccatgGTGAGCTCTG
VMH 333 Nsa5 RP	atcgatGAATTCGGTCCGGTAGTTGATGCTAATAGGAG
VMH 334 SHP2 FP	actagcGCTAGCaccatgacatcgcggagatgg
VMH 335 SHP2 593aa RP	cgatcgAAGCTTtctgaaacttttctgctgttgcatcag
VMH 336 SHP2 SH2 217 RP	gcactgAAGCTTgttaaggggctgcttgagttgtag
VMH 337 KpnI GFP -for SHP2 FP	atcgatGGTACCatggtgagcaagggcgag
VMH 338 EcoRI GFP- for SHP2 RP	actgacGAATTCttacttgtacagctcgtccatgcc

Supplemental Table 3. Primers used for cloning.