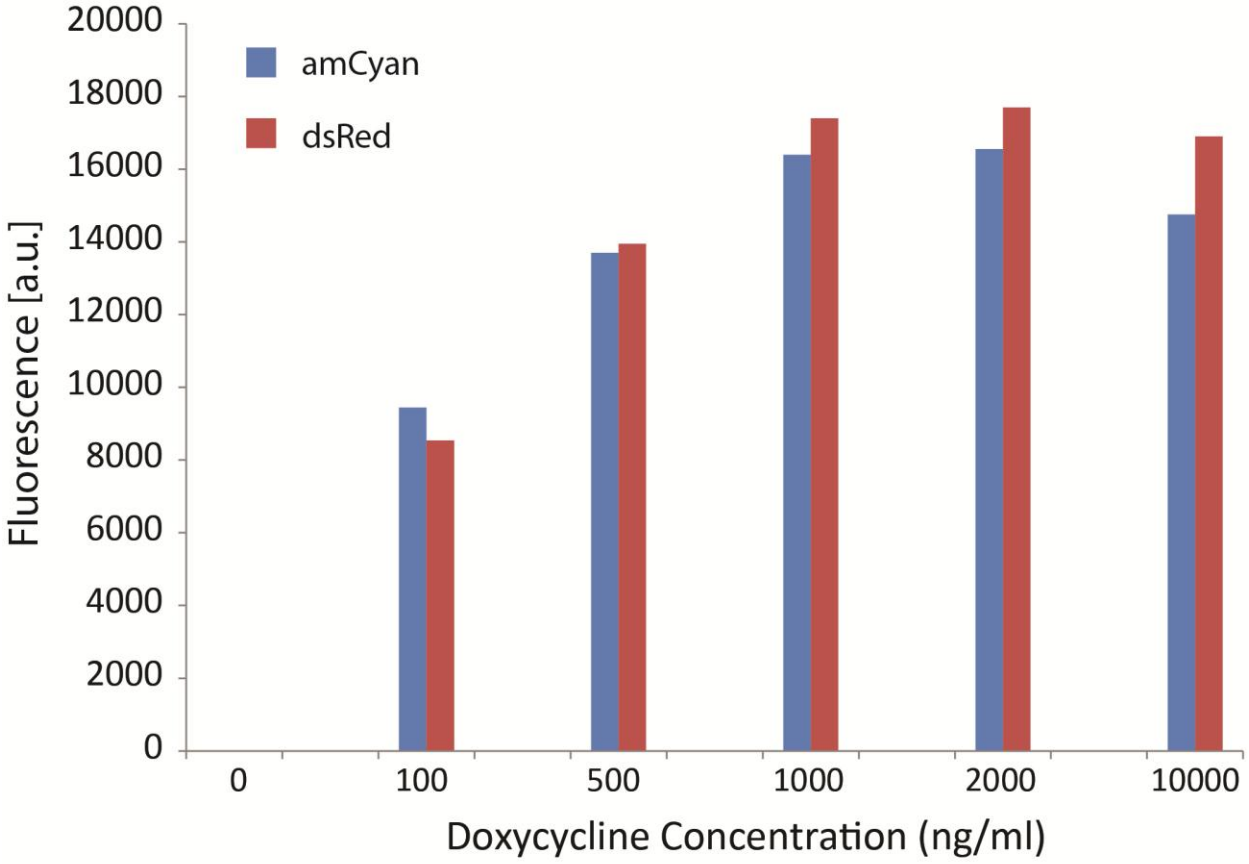
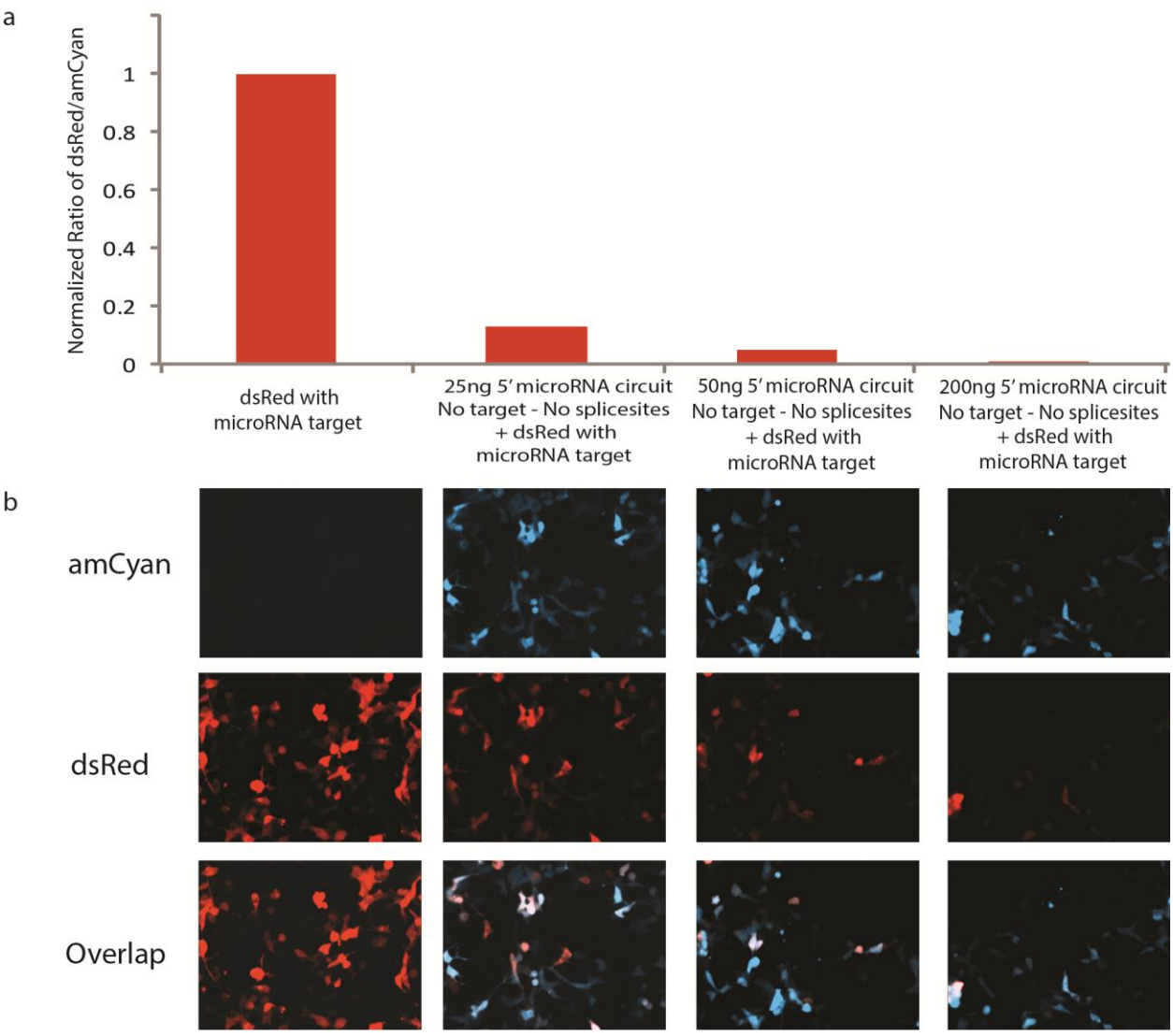
Supplementary Material

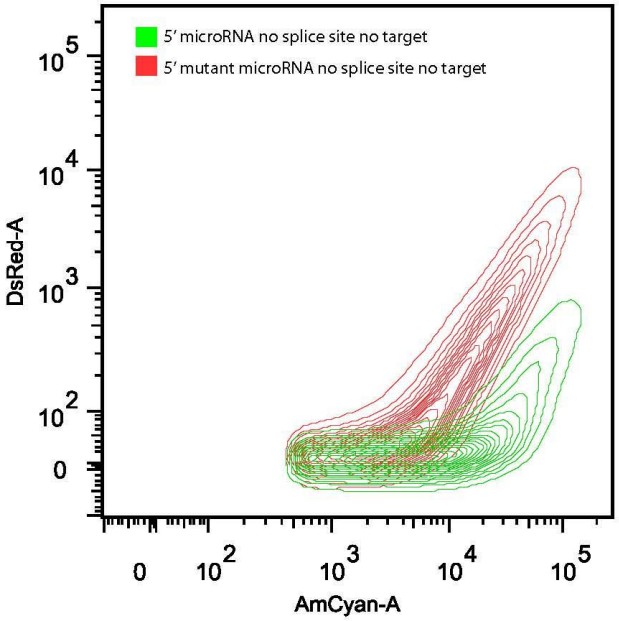
Supplementary Figures



Supplementary Figure 1 Dox titration. The Dox titration was performed with the intronic miR without target circuit. The amount of Dox was varied from 100ng/ml to 10,000ng/ml. Maximum expression was observed at 1000ng/ml concentration. 1000ng/ml Dox was used in all the main paper experiments.



Supplementary Figure 2 Control experiment for 5′UTR microRNA no splice sites. Titration was performed to investigate the processing of microRNA from the 5′ UTR microRNA circuit. The figure shows microscopy and flow cytometry results. A construct expressing dsRed with microRNA target in its 3′ UTR region was transfected along with the 5′ microRNA circuit in HEK293 TET-ON cells. The dose dependent inhibition of the dsRed protein confirms that the microRNA is processed correctly from the 5′ UTR microRNA circuits. Microscopy and flow cytometry data was collected after 48hrs.



Supplementary Figure 3 Overlaid density contour plots of the 5′UTR constructs without splice sites and with mutated (red) and normal (green) microRNAs.

Constructs

The backbone plasmid pTRE-Tight-Bi (Clontech) which contains a bidirectional pTRE-Tight promoter consisting of seven rtTA binding sites flanked by diverging minimal CMV promoters (CMV_{MIN}) and multiple cloning sites (MCS_I and MCS_{II}).

C1	pTRE-amCyan-dsRedexpress-no miRFF3-no FF3 target
C2	pTRE-amCyan-dsRedexpress-WT-miRFF3-splicesites-no FF3 target
C3	pTRE-amCyan-dsRedexpress-no miRFF3- with FF3 target
C4	pTRE-amCyan-dsRedexpress-5'-miRFF3-splicesites-no FF3 target
C5	pTRE-amCyan-dsRedexpress-3'-miRFF3-splicesites-no FF3 target
C6	pTRE-amCyan-dsRedexpress-WT-miRFF3-splicesites-with FF3 target
C7	pTRE-amCyan-dsRedexpress-5'-miRFF3-splicesites-with FF3 target
C8	pTRE-amCyan-dsRedexpress-3'-miRFF3-splicesites-with FF3 target
C9	pTRE-amCyan-dsRedexpress-WT-mutant miRFF3-splicesites-no FF3 target
C10	pTRE-amCyan-dsRedexpress-5'-mutant miRFF3-splicesites-no FF3 target
C11	pTRE-amCyan-dsRedexpress-3'-mutant miRFF3-splicesites-no FF3 target
C12	pTRE-amCyan-dsRedexpress-WT-mutant miRFF3-splicesites- with FF3 target
C13	pTRE-amCyan-dsRedexpress-5'-mutant miRFF3-splicesites-with FF3 target
C14	pTRE-amCyan-dsRedexpress-3'mutant miRFF3-splicesites-with FF3 target
C15	pTRE-amCyan-dsRedexpress-5'-miRFF3-no splicesites- no FF3 target
C16	pTRE-amCyan-dsRedexpress-3'-miRFF3-no splicesites- no FF3 target
C17	pTRE-amCyan-dsRedexpress-5'-miRFF3-no splicesites- with FF3 target
C18	pTRE-amCyan-dsRedexpress-3'-miRFF3-no splicesites- with FF3 target
C19	pTRE-dsRedexpress-no miRFF3-with target

Table 1: List of constructs

(C1) pTRE-amCyan-dsRedexpress-no miRFF3-no FF3 target: This is a plasmid obtained from Bleris et al, 2011. This is the backbone plasmid for most of our constructs without miRFF3 target

(C2) pTRE-amCyan-dsRedexpress-WT-miRFF3-splicesites-no FF3 target: This is a plasmid obtained from Bleris MSB 11.

MicroRNA-FF3 target

MicroRNA-FF3 target were designed complimentary to the microRNA-FF3 stem loop region. The sense and antisense (primers P1 and P2) oligos were synthesized from IDT. The oligos have a NheI and NotI sites and spacer base pairs on either ends to aid in digestion reaction. The oligos are diluted to a

concentration of 100pmol/µl. Annealing reaction is carried out with 250pmol each of sense and antisense primers and 0.5M NaCl in an annealing PCR reaction. The size of the oligo was tested in a metaphor gel and the PCR product was digested with NheI and NotI enzymes and gel purified.

(C3) pTRE-amCyan-dsRedexpress-no miRFF3- with FF3 target: This is the backbone plasmid for all our constructs with FF3 target in the 3'UTR of dsRedexpress.C1 is digested with HindIII and NotI to insert the FF3 target. The FF3 target was ligated in to the digested vector (C1) in the ratio of 4:1 at 16°C overnight, transformed and expanded and the integrity of the miRFF3-target was verified by sequencing by primer P3.

(C4) pTRE-amCyan-dsRedexpress-5'-miRFF3-splicesites-no FF3 target: This plasmid was built by amplifying the intron with the microRNA from construct C2 with Taq polymerase using primers P15 and P16 having BamHI and NheI enzyme sites. In parallel, the vector was prepared by digesting C1 plasmid with the same enzymes. The amplified insert was gel purified and digested. The vector and the insert were ligated in the ratio of 2:1 at 16°C overnight, then transformed and expanded. The sequence was verified with primer P8.

(C5) pTRE-amCyan-dsRedexpress-3'-miRFF3-splicesites-no FF3 target: For microRNA with splice sites at the 3' UTR, intron with the microRNA was amplified with Taq polymerase from C2 plasmid using primers P13 and P14 with HindIII and SalI restriction enzyme sites. The vector plasmid C1 and the PCR product is digested and ligated in the ratio of 2:1 at 16°C overnight, transformed and expanded. The correct sequence was confirmed by sequencing using primer P12.

(C6) pTRE-amCyan-dsRedexpress-WT-miRFF3-splicesites-with FF3 target: C2 is digested with NheI and NotI to insert the FF3 target. The FF3 target was ligated in to the digested vector (C2) in the ratio of 4:1 at 16°C overnight, transformed and expanded and the integrity of the miRFF3-target was verified by sequencing by primer P3.

(C7) pTRE-amCyan-dsRedexpress-5'-miRFF3-splicesites-with FF3 target: C4 is digested with NheI and NotI to insert the FF3 target. The FF3 target was ligated in to the digested vector (C4) in the ratio of 4:1 at 16°C overnight, transformed and expanded and the integrity of the miRFF3-target was verified by sequencing by primer P3.

(C8) pTRE-amCyan-dsRedexpress-3'-miRFF3-splicesites-with FF3 target: C5 is digested with NheI and NotI to insert the FF3 target. The FF3 target was ligated in to the digested vector (C5) in the ratio of 4:1 at 16°C overnight, transformed and expanded and the integrity of the miRFF3-target was verified by sequencing by primer P3.

(C9) pTRE-amCyan-dsRedexpress-WT-mutant miRFF3-splicesites-no FF3 target: To mutate the microRNA, the 22nts sequence involved in the stem loop formation was deleted and a random sequence was inserted which does not form a stem loops. The deletion and insertion step of microRNA was done by PCR. For the intronic mutant microRNA construct, primers were designed with mutated microRNA sequence and SpeI sites at the ends. The primers P17 and P18 were used to amplify the whole vector with the mutated miR sequence using plasmid C2 as the DNA template for the PCR reaction. The PCR products were digested with SpeI, ligated and transformed. Correct clones were identified by sequencing using primer P19.

(C10) pTRE-amCyan-dsRedexpress-5'-mutant miRFF3-splicesites-no FF3 target: For mutant microRNA at the 5' UTR, overlap extension PCR was used to amplify construct C4 using primers P21 and P22 having mutated miR sequence and primers P24, P25 to extend the PCR product to yield an amplicon which had the splice sites and the mutated miR having BamHI and NheI restriction sites at either ends. The PCR product and the plasmid vector C4 are digested with BamHI and NheI followed by ligation, transformation and expansion. The new constructs were sequenced using primer P8.

(C11) pTRE-amCyan-dsRedexpress-3'-mutant miRFF3-splicesites-no FF3 target: For mutant microRNA at the 3' UTR, overlap extension PCR was used to amplify construct C5 using primers P21 and P22 having mutated miR sequence and primers P20, P23 to extend the PCR product to yield an

amplicon which had the splice sites and the mutated miR. The primers P20 and P23 have HindIII and SalI restriction sites and digested following PCR. The plasmid backbone C5 is digested with HindIII and SalI to release the insert miRFF3 with splice sites and ligated with the final extension PCR product from the overlap PCR which has these restriction sites and the mutant miR. The new plasmids were sequenced using primer P10.

(C12) pTRE-amCyan-dsRedexpress-WT-mutant miRFF3-splicesites- with FF3 target: The primers P17 and P18 were used to amplify the whole vector with the mutated miR sequence using plasmid C6 as the DNA template for the PCR reaction. The PCR products were digested with SpeI, ligated and transformed. Correct clones were identified by sequencing using primer P19.

(C13) pTRE-amCyan-dsRedexpress-5'-mutant miRFF3-splicesites-with FF3 target: For mutant microRNA at the 5' UTR, overlap extension PCR was used to amplify construct C7 using primers P21 and P22 having mutated miR sequence and primers P24, P25 to extend the PCR product to yield an amplicon which had the splice sites and the mutated miR having BamHI and NheI restriction sites at either ends. The PCR product and the plasmid vector C7 are digested with BamHI and NheI followed by ligation, transformation and expansion. The new constructs were sequenced using primer P8.

(C14) pTRE-amCyan-dsRedexpress-3'mutant miRFF3-splicesites-with FF3 target: For mutant microRNA at the 3' UTR, overlap extension PCR was used to amplify construct C8 using primers P21 and P22 having mutated miR sequence and primers P20, P23 to extend the PCR product to yield an amplicon which had the splice sites and the mutated miR. The primers P20 and P23 have HindIII and SalI restriction sites and digested following PCR. The plasmid backbone C8 is digested with HindIII and SalI to release the insert miRFF3 with splice sites and ligated with the final extension PCR product from the overlap PCR which has these restriction sites and the mutant miR. The new plasmids were sequenced using primer P10.

(C15) pTRE-amCyan-dsRedexpress-5'-miRFF3-no splicesites- no FF3 target: For the microRNA at the 5' UTR, microRNA was amplified with Taq polymerase from C2 plasmid using primers P6 and P7

with BamHI and NheI sites. These enzyme sites are present in the 5’ UTR of the C1 plasmid and the PCR product is digested and ligated in the ratio of 2:1 at 16°C overnight, transformed and expanded. The correct sequence was confirmed by sequencing using primer P8.

(C16) pTRE-amCyan-dsRedexpress-3'-miRFF3-no splice sites- no FF3 target: For microRNA at the 3’ UTR, microRNA was amplified with Taq polymerase from C2 plasmid using primers P9 and P10 with HindIII and SalI restriction enzyme sites. These enzyme sites are present in the 3’ UTR of the C1 plasmid. The plasmid and the PCR product was digested and ligated in the ratio of 2:1 at 16°C overnight, transformed and expanded. The correct sequence was confirmed by sequencing using primer P12.

(C17) pTRE-amCyan-dsRedexpress-5'-miRFF3-no splice sites-with FF3 target: MicroRNA was amplified from C2 using primers P6 and P7 with BamHI and NheI sites. The vector used is the C3 plasmid which has the miRFF3 target at the 3’UTR. C3 plasmid and the PCR product is digested with BamHI and NheI and ligated in the ratio of 2:1 at 16°C overnight, transformed and expanded. The correct sequence was confirmed by sequencing using primer P8.

(C18) pTRE-amCyan-dsRedexpress-3'-miRFF3-no splice sites-with FF3 target: MicroRNA was amplified from C2 using primers P9 and P10 with HindIII and SalI restriction sites. The vector used is the C3 plasmid which has the miRFF3 target at the 3’UTR. C3 plasmid and the PCR product is digested with HindIII and SalI and ligated in the ratio of 2:1 at 16°C overnight, transformed and expanded. The correct sequence was confirmed by sequencing using primer P12.

(C19) pTRE-dsRedexpress-no miRFF3-with target: Construct C3 is digested with XbaI and EcorI to remove the amCyan from the plasmid.

Primers

P1	GGCCGCAAACGATATGGGCTGAATACAATACCA
P2	AGCTTGGTATTGTATTCAGCCCATATCGTTTGC
P3	CCCTGATTCTGTGGATAACC
P4	TTTGCTAGCGCTACCGGTCGCCACCA
P5	TTTGCGGCGGCCTACAGGAACAGGT
P6	TTTGGATCCGCTCGCTTCGGCAGCAC
P7	TTTGCTAGCCAATTGAAAAAAGTGATT
P8	GGAGATTTCGAGCTCGGTA
P9	TTTAAGCTTGCTCGCTTCGGCAGCAC
P10	TTTGTCGACCAATTGAAAAAAGTGATT
P11	ACCTTGAAGCGCATGAACTC
P12	CTCCAAGCTGGACATCACCT
P13	TTTAAGCTTGAGGTGAGTATGTGCTCGCTTCG
P14	TTTGTCGACGCGGCCCTGAGGAAAAAAAAGGAAAC
P15	TTTGGATCCGAGGTGAGTATGTGCTCGCTTCG
P16	TTTGCTAGCGCGGCCCTGAGGAAAAAAAAGGAAAC
P17	CAGCGACTAGTGAATTCACTTCCATATACGGTTTGCCTACTGCCTCGGAGAATTCAA
P18	AAGCGACTAGTCTGCAGCAGACTGAACTCATTGCGCTCACTGTCAACAGCACTCGAGC
P19	TTACAGAATCGTTGCCTGCAC
P20	CCAGTAAAGCTTGTGAGTATGTGCTCGCTTC
P21	ACCGTATATGGAAGTGAATTCACTAGTCTGCAGTTACTCAAGTCAGAGGCGCTCACTGTCAACAGCAC
P22	CTCTGACTTGAGTAACTGCAGACTAGTGAATTCACTTCCATATACGGTTTGCCTACTGCCTCGGAGAA
P23	CCAGTAGTCGACCTGAGGAAAAAAAAGGAAACA
P24	CCAGTAGGATCCGTGAGTATGTGCTCGCTTC
P25	CCAGTAGCTAGCCTGAGGAAAAAAAAGGAAACAA

Table 2: List of primers

MicroRNA FF3 sequence

ACGATATGGGCTGAATACAAATAGTGAAGCCACAGATGTATTTGTATTCAGCCCATATCGT

Mutated microRNA FF3 sequence

CTCTGACTTGAGTAACTGCAGACTAGTGAATTCACTTCCATATACGGT