

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Zinc-finger-luciferase constructs as genuine fusion proteins. In order to verify that the ORF-luciferase proteins were being appropriately expressed, firefly luciferase expression, normalized to the *Renilla* luciferase transfection control, of wild-type constructs was compared with that of mutant constructs where the reading frame was disrupted by a nucleotide insertion/deletion early in the coding region of the zinc-finger half of the fusion protein. Error bars represent the third largest and third smallest values ($n \leq 5$; $p < 0.005$).

Supplemental Figure 2. Repression of (A) *ZNF20* and (B) *RBAK* by miR-181a increased when expressed as a 3'UTR. Direct repression of *ZNF20* and *RBAK*, which was expressed as a 3'UTR, mediated by miR-181a, as assayed by luciferase assay. Fold repression was calculated relative to that of the non-cognate miRNA, miR-23a. Plotted are the normalized values where the repression of the reporter with wild-type sites (WT) was normalized to that of the mutant reporter in which the miR-181 sites were mutated. Error bars represent the third largest and third smallest values (for *ZNF20*, $n = 15$, $p < 10^{-7}$; for *RBAK*, $n=12$, $p < 10^{-4}$).

Supplemental Figure 3. miR-181a directly represses *RBAK* ORF and 3'UTR. The repression of *RBAK* ORF-luciferase fusion proteins followed by *RBAK* 3'UTR by miR-181a was assayed by luciferase assays. Fold repression was calculated relative to that of the non-cognate miRNA, miR-23a. Plotted are the normalized values where the repression of the reporter with wild-type sites (WT) was normalized to that of the mutant reporter in which the nineteen ORF and two 3'UTR sites were mutated. Error bars represent the third largest and third smallest values ($n = 12$; $p < 8 \times 10^{-7}$).

Supplemental Figure 4. The effect of miRNA duplexes upon firefly luciferase expression. Fold repression was calculated relative to that of the no duplex control. Plotted are the normalized values with error bars representing the third largest and third

smallest values ($n \leq 9$; $p = 0.04$ for miR-124; $p < 5 \times 10^{-6}$ for miR-199a; $p = 0.48$ for miR-370).

Supplemental Figure 5. The relationship between shared ancestry of KRAB-containing C₂H₂ genes and shared miRNA sites. As in Figure 4B, the phylogeny inferred from the alignment of the KRAB domains is shown, marking at the perimeter those with at least four 8mer sites to the indicated miRNA.

Supplemental Figure 6. Similarity of codons across C₂H₂ domains for real zinc-finger genes compared to those with randomized nucleotide sequences (see Methods). Shown are the fractions of equal codon sequences across all pairs of C₂H₂ domains within the same gene for each position in the C₂H₂ domain for both real sequences and randomized sequences. Error bars show the standard deviation from 50 codon randomization trials.

Supplemental Figure 7. Direct repression of *RB1* ORF mediated by miR-181a, as assayed by luciferase assays. Fold repression was calculated relative to that of the non-cognate miRNA, miR-23a. Plotted are the normalized values where the repression of the reporter with wild-type sites (WT) was normalized to that of the mutant reporter in which the three ORF sites were mutated. Error bars represent the third largest and third smallest values ($n=12$; $p=7 \times 10^{-7}$). No repression was observed for *RB1* 3'UTR ($n=12$; $p=0.93$).

SUPPLEMENTAL TABLES

Supplemental Tables 1-7. MicroRNA target sets (genes with 4 or more non-overlapping 8mer seed sites) for miR-23, miR-181, miR-188, miR-199, miR-370, miR-766 and miR-1248. Genes with ORFs of length > 10 kilobases were excluded from the analysis.

Supplemental Table 8. DNA oligonucleotides used in this study.

Name	Sequence
LinkerInsertF	CTAGCTGATGGGTACCGGCGGTTCTGGAGGG
LinerInsertR	GTGACCCCTCCAGAACCGCCGGTACCCATCAG
ControlPrimerF	ATAGGCTAGCCACCATGGTCACCGACGCCAAAAACATAAAG
ControlPrimerR	TGTTGAGCTCATTACACGGCGATCTTCCGCC
Znf573 Fw	GTTGAGCTAGCCACCATGACCTGTTTCAGGAATTAG
Znf573 Rev	CTTGAGGTACCCACTTTTATGCTCCTATGAATTC
Zfp37 Fw	GTTGAGCTAGCCACCATGTCGGTCTCCAGCGGC
Zfp37 Rev	CTTGAGGTACCCCTCATGAGATTATCTTCTGAATGAG
Znf20 Fw	GTTGAGCTAGCCACCATGATGTTTCAGGATTCAGTGG
Znf20 Rev	CTTGAGGTACCTCTATTAATGGTATGAGTTCTTTCA
Znf791 Fw	GTTGAGCTAGCCACCATGGACTCAGTGGCTTTTGAGG
Znf791 Rev	CTTGAGGTACCTCGATTGTGCATTCTCATATG
RBAK Fw	GTTGAGCTAGCCACCATGAACACATTGCAGGGGC
RBAK Rev	CTTGAGGTACCGAGATTTTCCACATCAAGTACATTC
Znf225 Fw	GTTGAGCTAGCCACCATGACCACGTTGAAGGAGG
Znf225 Rev	CTTGAGGTACCTGTGTCAATTTAAAAATAATGACAAA
Znf486 Fw	GTTGAGCTAGCCACCATGCCGGGACCCCTTAGAA
Znf486 Rev	CTTGAGGTACCCGTTCTTGGTTTCTGTCCAA
Znf85 Fw	GTTGAGCTAGCCACCATGAGCCTCAGCGCCAG
Znf85 Rev	CTTGAGGTACCTATTTGTAATTTTCTCCGGTATGA
Znf573_ins_1nt_after_622	CCCTAATCAGAGGGACTTATACACGGGATGTGATGT
Znf573_ins_1nt_after_622-antisense	ACATCACATCCCGTGTATAAGTCCCTCTGATTAGGG
Zfp37_ins_1nt_after_622	CGGGCGACCACTGGAGACTGGCTGTGT
Zfp37_ins_1nt_after_622-antisense	ACACAGCCAGTCTCCAGTGGTCGCCCCG
Znf20_ins_1nt_after_622	GAAGAATCTCTACAGGGCATGTGATGCAGGAAACC
Znf20_ins_1nt_after_622-antisense	GGTTTCCTGCATCACATGCCCTGTAGAGATTCTTC
Znf791_ins_1nt_after_622	CTCTACAGAGATGTGATGCCAGGAAACATTCAAGAACCT
Znf791_ins_1nt_after_622-antisense	AGGTTCTTGAATGTTTCCTGGCATCACATCTCTGTAGAG
RBAK_ins_1nt_after_622	GACCTGATGAGAAGATAACTTACACGGGATGTGATGTT
RBAK_ins_1nt_after_622-antisense	AACATCACATCCCGTGTAAAGTTATCTTCTCATCAGGGTC
Znf85_ins_1nt_after_754	GTAATGGACTTAACCAATCGTCTCACAGCTACCCAG
Znf85_ins_1nt_after_754-antisense	CTGGGTAGCTGTGAGACGATTGGTTAAGTCCATTAC
Znf20_a963g_t966c	AGAGAATCCATATAGAAATAAGGAGTGCAAGAAAGCCTTCAGTTATCTTGAC
Znf20_a1047g_t1050c	CTAAAGAGAAAACCTATGATGGTAAAGAGTGCACAGAAACCTTCATTTC
HDAC5_Forward	GTTGAGCTAGCCACCATGTCCAGCAACACACACTG
HDAC5_Reverse	CTTGAGGTACCTTTATGTTTGGGTGGCCACTGC
IVL_Forward	GTTGAGCTAGCCACCATGAACTCTCCCAACGATCG
IVL_Reverse	CTTGAGGTACCCAGGGCAGGCTCCTGCTC
RBAK 3'UTR Fw	CTTGAACCGGTAGTCAGATCTCAATTTTGTAGAAAACCTCTTGAA
RBAK 3'UTR Rev	GTTGGCGGCCGCTCCAGCAAGAAATGGAGCGAG
RBAK UTR site 1	CACAGAGAAGAATCCCGAAGTTTGTAAACAAGAAGCAAAGCCT
RBAK UTR site 2	CAAAGGCAAAATCTGTCAATATGGTGTGTTGTGGAAAATATATTGT
	CTTGGAAT
Znf20_Into3UTR_Forward	GTTGATCTAGAATGATGTTTCAGGATTCAGTGG
Znf20_Into3UTR_Reverse	CTTGAGCGGCCGCTCTATTAATGGTATGAGTTCTTTCA
RB1_ORF_For	GTTGAACCTAGTCACCATGCCGCCCAAAACCCC
RB1_ORF_Rev	CTTGAGGTACCTTTCTCTTCCTGTTTGGAGGTATCCAT
RB1_UTR_For	CTTGAACCGGTGGATCTCAGGACCTTGGTGGA
RB1_UTR_Rev	GTTGGCGGCCGCTGTAGAAAATAGTAACATAGCAATTTTAAATGTACAGTT
RB1_a1368g_t1371c	AATTATTGAAGTTCTCTGTAAAGAACATGAGTGCAATATAGATGA
	GGTGAATAATGTTTATTTC

RB1_g1620c_t1623c	GTAACCTTGATGAAGAGGTCAACGTAATTCCTCCACACACTC
RB1_g2076c_t2079c	AGATTTGTCTTTCCCATGGATTCTCAACGTGCTTAATTTAAAAGCCTTTGAT
RB1_a5691g_t5694c	CTACTGAAACAGATTTTCATACCTCAGAGTGCAAAAGAACTTACTG
	ATTATTTTCTTCA
RB1_a5691g_t5694c_antisense	TGAAGAAAATAATCAGTAAGTTCTTTTGCACTCTGAGGTATGAA
	ATCTGTTTCAGTAG
RBAK_a4543g_t4546c	GGACAAAACCTGATGAGTGTAATGAGTGCGGGAAAACATATCATGGAG
RBAK_a4837g_t4840c	GAAATGAAGCCCTTTGAATGCAGTGAGTGCGGAAAATCCTTCTGTAAA
RBAK_a5005g_t5008c	TAGAGGAGAAGCCCTATAAATGTAATGAGTGCGGGAAAACCTTTTGTC
RBAK_a4912g_t4915c	CACAGGAGAGAAAACCTTATGAGTGCAATGTATGTGGGAAATCCTTC
RBAK_a5080g_t5083c	CATTTCAGGAGAGAAAACCTACGAGTGCAGCGAATGTGGG
RBAKintoUTR_Forward	GTTGATCTAGAGTGATGGAAGCTATGCTAGGACA
RBAKintoUTR_Reverse	CTTGAGCGGCCGCCCTCCAGAACCGCCGGTA
IVL_g774a_g777a	GAAGGAGCCACAGGAACAAGAGCTGCAGCAACAG
IVL_g849a_g852a	AACCCAGAGCAGCAGCTTAAACAAGAGAAAACACAAAGGGA

Supplemental Table 9. RNA oligonucleotides used in this study.

Name	Sequence
miR-23a	AUCACAUUGCCAGGGAUUUCC
miR-23a Passenger Strand	AAAUCCCUGGGGAUGGGAUUU
miR-124	UAAGGCACGCGGUGAAUGCCA
miR-124 Passenger Strand	GCAUUCACCGCGUGCCUUAU
miR-181a	AACAUUCAACGCUGUCGGUGAGU
miR-181a Passenger Strand	UCACCGACAGCGUUGAAUGAUU
miR-199a	CCCAGUGUUCAGACUACCUGUUC
miR-199a Passenger Strand	ACAGGUAGUCUGAACACUGGGUU
miR-370	GCCUGCUGGGGUGGAACCUGGU
miR-370 Passenger Strand	CAGGUUCCACCCCAGCAGGCUU

Supplemental Table 10. Plasmids used in this study.

Name	Description
pIS1	Control <i>Renilla</i> luciferase construct
pIS7L	Empty vector firefly luciferase construct
pIS7L-Znf573	<i>ZNF573</i> -luciferase fusion construct
pIS7L-Zfp37	<i>ZFP37</i> -luciferase fusion construct
pIS7L-Znf20	<i>ZNF20</i> -luciferase fusion construct
pIS7L-Znf791	<i>ZNF791</i> -luciferase fusion construct
pIS7L-RBAK	<i>RBAK</i> ORF-luciferase fusion construct
pIDTSMART-mZnf20	Minigene construct with half of Znf20 mutated
pIS7L-mZnf20	<i>ZNF20</i> -luciferase fusion construct with miR-181 sites mutated
pIS7L-Znf20-in-UTR	Luciferae followed by <i>ZNF20</i> in 3'UTR
pIS7L-mZnf20-in-UTR	Luciferae followed by <i>ZNF20</i> in 3'UTR with miR-181 sites mutated
pIS7L-RBAK-in-UTR	Luciferae followed by <i>RBAK</i> in 3'UTR
pIS7L-mRBAK-in-UTR	Luciferae followed by <i>RBAK</i> in 3'UTR with miR-181 sites mutated
pIS7L-fs Znf573	<i>ZNF573</i> -luciferase fusion construct with frame-shift
pIS7L-fs Zfp37	<i>ZFP37</i> -luciferase fusion construct with frame-shift
pIS7L-fs Znf20	<i>ZNF20</i> -luciferase fusion construct with frame-shift
pIS7L-fs Znf791	<i>ZNF791</i> -luciferase fusion construct with frame-shift
pIS7L-fs RBAK	<i>RBAK</i> ORF-luciferase fusion construct with frame-shift
pIS7L-Znf225	<i>ZNF225</i> -luciferase fusion construct
pIS7L-Znf486	<i>ZNF486</i> -luciferase fusion construct
pIS7L-Znf85	<i>ZNF85</i> -luciferase fusion construct
pIS7L-fs Znf85	<i>ZNF85</i> -luciferase fusion construct with frame-shift
pIS7L-HDAC5	<i>HDAC5</i> -luciferase fusion construct
pIS7L-IVL	<i>IVL</i> -luciferase fusion construct
pIS7L-mIVL	<i>IVL</i> -luciferase fusion construct with miR-370 sites mutated
pIDTSMART-mIVL1	Minigene construct with half of <i>IVL</i> mutated
pIDTSMART-mIVL2	Minigene construct with half of <i>IVL</i> mutated
pIDTSMART-mRBAK	Minigene construct with half of <i>RBAK</i> mutated
pIS7L-mRBAK	<i>RBAK</i> ORF-luciferase fusion construct with miR-181 sites mutated
pIS7L-RBAK UTR	Luciferase followed by <i>RBAK</i> 3'UTR construct
pIS7L-RBAK mUTR	Luciferase followed by <i>RBAK</i> 3'UTR construct with miR-181 sites mutated
pIS7L-RBAK O+U	<i>RBAK</i> -luciferase fusion followed by <i>RBAK</i> 3'UTR construct
pIS7L-RBAK mO+U	<i>RBAK</i> -luciferase fusion followed by <i>RBAK</i> 3'UTR construct with miR-181 sites mutated
pIS7L-Rb1	<i>RBI</i> -luciferase fusion construct
pIS7L-mRb1	<i>RBI</i> -luciferase fusion construct with miR-181 sites mutated
pIS7L-Rb1 UTR	Luciferase followed by <i>RBI</i> 3'UTR construct
pIS7L-Rb1 mUTR	Luciferase followed by <i>RBI</i> 3'UTR construct with miR-181 sites mutated

SUPPLEMENTAL MATERIALS AND METHODS

Plasmid construction.

pIS7L. In order to generate the control empty vector luciferase plasmid, we first amplified the firefly luciferase from pSP-*luc*+NF fusion vector (Promega) with ControlPrimerF and ControlPrimerR. The resulting product and pIS1 (Grimson et al. 2007) was with NheI and SacI and ligated to create pIS7. This construct was then digested with NheI and BstEII and ligated with an annealed duplex of LinkerInsertF and LinkerInsertR to generate pIS7L.

C-Terminal Luciferase Fusions. In order to generate C-terminal luciferase fusions, the following ORFs (*ZNF573*, *ZFP37*, *ZNF20*, *ZNF791*, *RBAK*, *ZNF225*, *ZNF486*, *ZNF85*, *HDAC5* and *IVL*) were amplified from cDNA clones (BC042170, BC126390, BC036714, BC106938, BC136676, BC108912, BC117268, BC051824 and BC046391, respectively) with the following oligonucleotides: Znf573 Fw, Znf573 Rev; Zfp37 Fw, Zfp37 Rev; Znf20 Fw, Znf20 Rev; Znf791 Fw, Znf791 Rev; RBAK Fw, RBAK Rev; Znf225 Fw, Znf225 Rev; Znf486 Fw, Znf486 Rev; Znf85 Fw, Znf85 Rev; HDAC5_Forward, HDAC5_Reverse; IVL_Forward, IVL_Reverse. The resulting products and pIS7L were digested with KpnI and NheI in order to generate the luciferase fusion constructs. To generate pIS7L-RB1, the *RB1* ORF was amplified from the cDNA clone BC039060 genomic DNA with RB1_ORF_Fw and RB1_ORF_Rev. The resulting product was digested with KpnI and SpI and ligated into pIS7L, which had been digested with KpnI and NheI.

Frame-shift mutant luciferase fusions. In order to generate frame-shifts (1 nucleotide insertion about 100 bp downstream of the start codon), QuikChange II (Stratagene) was used, following the manufacturer's instructions and the following oligonucleotides:

ZNF573: Znf573_ins_1nt_after_622; Znf573_ins_1nt_after_622-antisense
ZFP37: Zfp37_ins_1nt_after_622; Zfp37_ins_1nt_after_622-antisense
ZNF20: Znf20_ins_1nt_after_622; Znf20_ins_1nt_after_622-antisense
ZNF791: Znf791_ins_1nt_after_622; Znf791_ins_1nt_after_622-antisense
RBAK: RBAK_ins_1nt_after_622; RBAK_ins_1nt_after_622-antisense
ZNF85: Znf85_ins_1nt_after_622; Znf85_ins_1nt_after_622-antisense

ZNF20 mutant ORF luciferase fusion. Using QuikChange-Multi kit (Stratagene) and two oligonucleotides (Znf20_a963g_t966c, Znf20_a1047g_t1050c), two 7mers were mutated to generate pIS7L-Znf20int. The *ZNF20* minigene (see below) was excised from pIDTSMART-mZnf20 by StuI digested. This was ligated into pIS7L-Znf20int, which had also been digested by StuI, to generate pIS7L-mZnf20.

RBAK mutant ORF luciferase fusion. The N-terminal section of *RBAK* was excised from pIS7L-RBAK using HindIII. This fragment was ligated into digested pIS0 (Grimson et al. 2007) to generate pRBAKPreMutate. To generate pRBAKMutate1, pRBAKPreMutate was then mutated using QuikChange-Multi (Stratagene) and the following oligonucleotides: RBAK_a4543g_t4546c, RBAK_a4837g_t4840c and RBAK_a5005g_t5008c. To generate pRBAKMutate2, pRBAKMutate1 was mutated

using QuikChange Multi and the following oligonucleotides: RBAK_a4912g_t4915c and RBAK_a5080g_t5083c. In order to generate pRBAKInterim, the *RBAK* minigene (see below) was excised using HindIII and KpnI and ligated into pIS7L-RBAK, also digested with HindIII and KpnI. Finally, to generate pIS7L-mRBAK, the mutated N-terminal half of RBAK was excised from pRBAKMutate2 with HindIII and ligated into pRBAKInterim, which had also been digested with HindIII.

ZNF20 and RBAK into 3'UTR luciferase construct. Wild-type or mutant *ZNF20* was amplified with Znf20_Into3UTR_Forward and Znf20_IntoUTR_Reverse; wild-type or mutant *RBAK* was amplified with RBAKintoUTR_Forward and RBAKintoUTR_Reverse. The resulting products as well as pIS7L were digested with XbaI and NotI to generate pIS7L-Znf20-in-UTR and pIS7L-mZnf20-in-UTR, respectively. *RB1 mutant ORF luciferase fusion.* pIS7L-Rb1 was mutated using QuikChange Multi kit (Stratagene), according to manufacturer's instructions, and the following oligonucleotides: RB1_a1368g_t1371c, RB1_g1620c_t1623c and RB1_g2076c_t2079c.

IVL mutant ORF luciferase fusion. To mutate the two 7mer sites, pIS7L-IVL was first mutated with IVL_g774a_g777a and IVL_g849_g852a to generate pIS7L-IVLint1. This plasmid was then digested with PflmI and DraIII; IVL minigene1 was similarly digested, and the resultant 800 bp fragment was ligated into digested pISL-IVLint1 to generate pIS7L-IVLint2. IVL minigene2 was excised using DraIII and KpnI, and ligated into pIS7L-IVLint2 similarly digested to give rise to pIS7L-mIVL.

3'UTR Luciferase Constructs. The *RBAK* 3'UTR was amplified from the cDNA clone BC136676 using the oligonucleotides *RBAK* 3'UTR Fw/Rev. The resulting product was digested with AgeI and NotI and ligated into digested pIS7L or pIS7L-RBAK in order to generate pIS7L-RBAK UTR or pIS7L-RBAK O+U, respectively. In order to generate pIS7L RBAK mUTR, QuikChange Multi kit (Stratagene) was used to mutate the original, wild-type plasmid with the oligonucleotides RBAK UTR site 1 and RBAK UTR site 2. The resulting mutant UTR was excised with AgeI and NotI and ligated into digest pIS7L-mRBAK in order to generate pIS7L-RBAK mO+U.

The *RB1* 3' UTR was amplified from the cDNA clone BC039060 using the oligonucleotides RB1_UTR_For and RB1_UTR_Rev. The resulting product was digested with AgeI and NotI. In order to mutate the single miR-181 site, QuikChangeII mutagenesis kit was used and the oligonucleotides RB1_a5691g_t5694c and RB1_a5691g_t5694c_antisense.

Minigenes

ZNF20. Generated in pIDTSMART (IDT Technologies) and flanked by StuI restriction sites. Mutated sites are in lower-case.

```
TTACTCGTTCCACTACCCCTTCCAGTACATGAAAGAACTCACACAGGAGTGAAT
GCCGAtgagtgcAAGAATGTGGGAATGCATTTCAGTTTTCCTAGTGAAATTCGTA
GACATAAAAGGTCTCACACTGGAGAAAAACCCTATGAGTGTAAGCAATGTGG
GAAAGTCTTCATTTCTTTCAGTTCCATTCAGTATCATAAGATGACTCACACTG
GAGAGAAACCCTAtgagtgcAGCAGTGTGGGAAAGCCTTTAGATGTGGCTCACA
CCTTCAAAAGCATGGAAGGACTCACACTGGAGAGAAACCCTAtgagtgcAGCAA
```

TGTGGTAAAGCCTTCAGATGTACCTCGGACCTTCAAAGGCATGAAAAGACAC
ACACTGAGGATAAACCCCTATGGATGTAAGCAGTGTGGGAAAGGCTTTAGATG
TGCTTCACAACCTTCAAATTCATGAAAGGACGCACAGTGGAGAGAAACCCCAtg
agtgaAGGAATGTGGAAAAGTATTCAAGTATTTTCTTCCTTGCGTATACATGA
AAGGACGCACACTGGAGAGAAGCCCCAtgagtgaAGCAATGTGGAAAAGCATT
CAGGTATTTCTCTTCCTTG CATATACATGAAAGGACACACACTGGAGATAAG
CCATATGAGTGTAAAGGTATGTGGCAAAGCCTTCACTTGTTCCAGTTCCATTG
ATATCATGAAAGGACTCACACTGGAGAGAAACCCTAtgagtgaAGCACTGTGGT
A

RBAK. Generated in pIDTSMART (IDT Technologies) and flanked by HindIII (5' side) and KpnI (3' side). Mutated sites are in lower-case.

TATAAATGTAATGAgTGcGGGAAATCCTACTACCGAAAGTCTACTCTGATTAC
ACATCAGAGAACACACACAGGAGAGAAGCCCTATCAGTGTAGCGAGTGTGG
GAAATTCTTTTCTCGGGTGT CATACCTCACTATACATTATAGAAGTCATTTAG
AAGAGAAACCCTATGAgTGcAATGAgTGcGGCAAACCTTCAATTTAAATTCA
GCCTTCATTAGACATCGGAAAGTACACACAGAAGAGAAATCCCATGAgTGcA
GTGAgTGcGGAAAGTTCTCTCAGTTGTATCTCACCGACCATCATACAGCTCATT
TAGAAGAGAAACCCTATGAgTGcAATGAgTGcGGGAAACCTTCCTTGTAAT
TCAGCCTTCGATGGGCACCAGCCACTTCCAAAAGGGGAGAAATCCTATGAgT
GcAATGTATGTGGAAAGTTATTCAATGAGTTGTCATACTATACTGAACATTAT
AGAAGTCATT CAGAAGAGAAACCTTATGGATGTAGCGAATGTGGGAAAACCT
TTTCCCATAATTCATCCCTCTTCAGACATCAAAGAGTACACACAGGCGAGAA
ACCCTATGAgTGcTACGAATGTGGAAAATTCTTCTCTCAGAAATCATATCTCA
CTATACATCATCGAATTCATT CAGGAGAGAAACCCTATGAgTGcAGTAAATGT
GGAAAAGTCTTCTCTCGGATGTCAAACCTCACTGTCCACTACAGAAGCCATTC
AGGAGAGAAACCCTATGAgTGcAATGAgTGcGGGAAAGTCTTTTCTCAGAAGT
CATACCTCACTGTACACTATAGAACTCATT CAGGAGAGAAACCCTATGAgTGc
AATGAGTGTGGGAAAAAATTCCACCACAGATCAGCCTTCAATAGCCATCAGA
GAATTCATAGAAGAGGAAATATGAAcGTcCTTGATGTGGAAAATCTC

IVL 1. Generated in pIDTSMART (IDT Technologies). Mutated sites are in lower-case.

CCAGCAACTGGATCAAGAGCTAGTCAAGAGAGATGAGCAACTGGGAATGAA
GAAAGAGCAACTGTTGGAGCTCCCAGAGGcaacaagaGGGGCACCTGAAGCACCT
AGAGGcaacaagaGGGACAGCTGAAGCACCCGGAGGcaacaagaGGGGCAGCTGGAGCT
CCCAGAGGcaacaagaGGGGCAGCTGGAGCTCCCAGAGGcaacaagaGGGGCAGCTGGA
GCTCCCAGAGGcaacaagaGGGGCAGCTGGAGCTCCCAGAGGcaacaagaGGGGCAGCT
GGAGCTCCCACAGGcaacaagaGGGGCAGCTGGAGCTCTCTGAGcaacaagaGGGGCA
GCTGGAGCTCTCTGAGcaacaagaGGGGCAGCTGGAGCTCTCTGAGcaacaagaGGG
ACAGCTGAAGCACCTGGAGCACCAGGAGGGGCAGCTGGAGGTCCCAGAGGA
GCAGATGGGGCAGCTGAAGTACCTGGAAcaacaagaGGGGCAGCTGAAGCACCT
GGATcaacaagaGAAGCAGCCAGAGCTCCCAGAGCAGCAGATGGGGCAGCTGAA
GCACCTGGAGGcaacaagaGGGGCAGCCTAAGCATCTGGAGGcaacaagaGGGGCAACT
GGAGCAGCTGGAGGcaacaagaGGGGCAGCTGAAGCACCTGGAGGcaacaagaGGGGC
AGCTGGAGCACCTGGAGCACCAGGAAGGGCAGCTGGGGCTCCCAGAGGcaacaag

TGCTGCAGCTGAAGCAGCTAGAGAAGGcaacaagGGCAGCCAAAGCACCTGGAGG
AGGAGGAGGGGCAGCTGAAGCACCTGGTG

IVL 2. Generated in pIDTSMART (IDT Technologies) and flanked by Kpn1 (3' side).

Mutated sites are in lower case.

CACCTGGTGcaacaagaGGGGCAGCTGAAGCATCTGGTGcaacaagaGGGGCAGCTG
GAGcaacaagaGAGGCAGGTGGAGCACCTGGAGcaacaagTGGGGCAGCTGAAGCA
CCTAGAGGaacaagaGGGACAAGTGAAGCATCTGGAGCAGcaacaagGGCAGTTGG
AGGTCCCAGAGcaacaagTGGGGCAGCCAAAGAACCTGGaacaagaGGAGAAGCAA
CTGGAGCTCCCAGAGCAGCAAGAGGGCCAGGTGAAGCACCTGGAGAAcaacaagaG
GCACAGCTGGAGCTCCCAGAGcaacaagTAGGACAGCCAAAGCACCTGGAAcaaca
agaAAAGCACCTAGAGCACCCAGAGcaacaagaCGGACAAGTAAAACATCTGGAG
caacaagaGGGGCAGCTGAAGGACCTGGAGCAGCAGAAGGGGCAGCTGGAGCAG
CCTGTGTTTGCCCCAGCTCCAGGCCAGGTCCAAGACATTCAACCAGCCCTGCC
CACAAAGGGAGAAGTATTGCTTCCTGTAGAGCACCAGCAGCAGAAcaacaagaGGT
GCAGTGGCCACCCAAACATAAA

SUPPLEMENTAL REFERENCES

Grimson, A., Farh, K.K., Johnston, W.K., Garrett-Engele, P., Lim, L.P., and Bartel, D.P.
2007. MicroRNA targeting specificity in mammals: determinants beyond seed
pairing. *Mol Cell* **27**(1): 91-105.