

Additional file 3: Supplementary data. Primers, probe, DNA sequences and sgRNA information.

Table 1. Primers and probe

miRNA/Gene	Primer	Sequence (5'-3')	Details
>hsa-miR-210-3p MIMAT0000267 CUGUGCGUGUGACA GCGGCUGA	RT primer	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGG CAATTTTTTTTTTTCAGCCG	miRNA qRT-PCR
	Forward primer	GGCTGTGCGTGTGACAGC	
>hsa-miR-21-5p MIMAT0000076 UAGCUUAUCAGACU GAUGUUGA	RT primer	CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGG CAATTTTTTTTTTCAACAT	
	Forward primer	CTCGGTAGCTTATCAGACTG	
>hsa-miR-26a-5p MIMAT0000082 UUCAAGUAAUCCAG GAUAGGCU	RT primer	GTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTAGCCTA	
	Forward primer	CCGGGTCAAGTAATCCAGGA	
SNORD44 NR_002750.2	RT primer	GTGCAGGGTCCGAGGTCAGAGCCACCTGGGCA ATTTTTTTTTTAGTCAG	
	Forward primer	CATGAAGGTCTTAATTAGCTCTA	
	Universal reverse primer	CAGTGCAGGGTCCGAGGT	mRNA qRT-PCR
	Universal Taqman probe	56-FAM/CAGAGCCAC/ZEN/CTGGGCAATTT/3I ABkFQ	
hNFAT5	hNFAT5 F1	CTCCTCAGATTCAGTTGGTACA	
	hNFAT5 R1	GACTGTTGCATATCTTGTGAT	
hTauT	hTauT-F	AGATCATCATAGGCCAGTACAC	
	hTauT-R	TAGACATTCAGGAGGGACACA	
hSMIT	hSMIT-F	GGCAGCCTATCTCTTCCATATC	
	hSMIT-R	GAGGGACCCTAATTCTCCAAAC	
β -actin	β -actin-F	AAAGACCTGTACGCCAACAC	sgRNA <i>in vitro</i> synthesis
	β -actin-R	GTCATACTCTGCTTGCTGAT	
NFAT5	T7-sg-1-F	TTCTAATACGACTCACTATAGCAAGTCAGTCAAA TTCAGAGGTTTTAGAGCTAGA	
	T7-sg-2-F	TTCTAATACGACTCACTATAGTGGTATGAGTGG AGTCATCAGTTTTAGAGCTAGA	
	T7-sg-3-F	TTCTAATACGACTCACTATAGAGTGCATTGTCTA CCAATGGTTTTAGAGCTAGA	
	T7-sg-4-F	TTCTAATACGACTCACTATAGATATGTTGGTCAT GATAGGGGTTTTAGAGCTAGA	
RFP	T7-sg-NC-F	TTCTAATACGACTCACTATAGCCACAGACTGGA AAGAATCAGTTTTAGAGCTAGA	
	Universal-sgRNA- R	AAAAAGCACCGACTCGGTGCCACTT	

MCS-PPGK-TetON3G -P2A-Puro	Tet2A-Puro-F	CATGATCGATACGCGTGAATTCCTCGAG	
	Tet2A-Puro-R	ACTAGTTCAGGCACCGGGCTTGCGGGT	
Luc	Luc-F	AAGAATGCGGCCGCATGGAAGATGCCAAAAAC ATTAAG	
	Luc-R	GATGACGCGTCGTCTCTCTAGATTACACGGCGA TCTTGCCGCCCTT	
rtTA3	rtTA3-F	TGCATTGAAATGTCTAGGCTGGACAAGAGCA	
	rtTA3-R	ATATCCCGGGGAGCATGTCAAGGTCA	
P _{CMV}	CMV-F	CATGCTAGATATTAATAGTAATCAATTACG	
	CMV-R	TGCATTGGAAGCCAGTAAGCAGTGGGTCTC	
P _{EF1α}	EF1α-F	CATGCTAGAAAGGATCTGCGATCGCTCCG	
	EF1α-R	TGCATTGGAACACAGCTTGATCTGTAACG	
P _{SV40}	SV40-F	CATGCTAGAGCGCAGCACCATGGCCTGA	
	SV40-R	TGCATTGGAAGCTTTTGCAAAAGCCTAG	
P _{ubc}	Ubc-F	CATGCTAGAGGCTCCGCGCCGGGTTTTG	
	Ubc-R	TGCATTGAAATGGATCCGCTAGCGTCTAAC	
EGFP	EGFP-F1	AAGAATGCGGCCGCATGGTGAGCAAGGGCGA	miRNA vector
	EGFP-R1	GATGACGCGTTTACTTGTACAGCTCGTCC	
EGFP	EGFP-F2	CATCGGATCCATGGTGAGCAAGGGCGA	cas9 vector
	EGFP-R2	CATGACTAGTTTACTTGTACAGCTCGTCC	
pri-hsa-miR-210	miR-210-F	GATGACGCGTCTGAAGTTGGGCCGAGAG	Primary miRNA
	miR-210-R	CATGATCGATGTATCTGGCCAGCCTCA	
pri-hsa-miR-21	miR-21-F	GATGACGCGTCCTTTAGGAGCATTATGAGC	
	miR-21-R	CATGATCGATATCCTCCCTCCATACTGCTG	
pri-hsa-miR-16-2	miR-16-F	GATGACGCGTCCTTAAAGTACTGTAGCAGCACA T	
	miR-16-R	CATGATCGATGGTAAATCAAACACCAAGTGTA CAG	
pri-hsa-miR-26a-2	miR-26a-F	GATGACGCGTCAGAAGCAAGCACAAGATCGG	
	miR-26a-R	CATGATCGATACCAGTTACACAGCAGCAAGG	
pri-hsa-miR-32	miR-32-F	GATGACGCGTCACTATCTCATTGAAGTTTTG	
	miR-32-R	CATGATCGATGATTTACTAAATGATCATTGC	
Cas9-FLAG-P2A-Puro	Cas9-2A-puro-F	AAGAATGCGGCCGCATGGACAAGAAGTACAGC ATCG	
	Cas9-2A-puro-R	GATGACGCGTTCAGGCACCGGGCTTGCGGGT	
NFAT5	T7E1-sg-1-F	ATCCTGATGCCTGTGAACCTGAGCT	T7E1 assay
	T7E1-sg-1-R	GCACATAAGCGGTAGTCAATGCATGG	
	T7E1-sg-2-F	TTCCAGAGGTCCCCAGCCAATGCA	
	T7E1-sg-2-R	GGCTCCTCAGTAACATTACAGGGGATAAGA	
	T7E1-sg-3-F	CCAGATGGTAATGAGAATGTTCAAG	
	T7E1-sg-3-R	GGAGAAGAAAACAATTCACACTGCA	
	T7E1-sg-4-F	CCAGATGGTAATGAGAATGTTCAAG	
	T7E1-sg-4-R	GAGGTTGATTGTCCCACTATGTTGCA	

Luciferase	Tet-5956-luc F1	TACACCTTCGTGACTTCCCATTTGC	qPCR
	Tet-5956-luc R1	GCATGACTGAATCGGACACAAG	

Sequence of artificially synthesized DNA

Note: Tet operators are underlined. TFIIB is shaded in grey. TATA box is indicated in red. Predicted initiator (Inr) is shaded in grey and underlined. Restriction sites are displayed in lower case.

(1) TRE3Gs

tctagacgtctcacgcgtTTTACTCCCTATCAGTGATAGAGAACGTATGAAGAGTTTACTCCCTATCAGTGATAGAGA
ACGTATGCAGACTTTACTCCCTATCAGTGATAGAGAACGTATAAGGAGTTTACTCCCTATCAGTGATAGAGAA
CGTATGACCAGTTTACTCCCTATCAGTGATAGAGAACGTATCTACAGTTTACTCCCTATCAGTGATAGAGAACG
TATATCCAGTTTACTCCCTATCAGTGATAGAGAACGTATAAGCTTTGCTTATGTAAACCAGGGCGCCTATAAAA
GAGTGCTGATTTTTTGAGTAAACTTCAATTCCACAACACTTTTGTCTTATACCAACTTTCCGTACCACTTCCTA
CCCTCGTAAAgcgccgc

(2) TRE3Gp

tctagacgtctcacgcgtTTTACTCCCTATCAGTGATAGAGAACGTATGAAGAGTTTACTCCCTATCAGTGATAGAGA
ACGTATGCAGACTTTACTCCCTATCAGTGATAGAGAACGTATAAGGAGTTTACTCCCTATCAGTGATAGAGAA
CGTATGACCAGTTTACTCCCTATCAGTGATAGAGAACGTATCTACAGTTTACTCCCTATCAGTGATAGAGAACG
TATATCCAGTTTACTCCCTATCAGTGATAGAGAACGTATAAGCTTTAGGCGTGTACGGTGGGCGCCTATAAAA
GCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGCAATTCCACAACACTTTTGTCTTATACgcgccgc

(3) TetO6

tctagacgtctcacgcgtTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAA
CGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAAC
GTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGTA
TGTCGAGGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCgcg
ccgc

(4) MCS-P_{PGK}-TetON3G-P2A-Puro

atcgatacgctGAATTCCTCGAGACCGGTgcggccgctctagaGGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCC
TGGGTTTGGCGAGGGACGCGGCTGCTCTGGGCGTGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTC
TCGCACATTCTTCAGTCCGTTTCGAGCGTCACCCGGATCTTCGCCGCTACCCTTGTTGGGCCCCCGGCGAC
GCTTCCTGCTCCGCCCTAAGTCGGGAAGGTTCTTGCGGTTTCGCGGCGTGCCGGACGTGACAAACGGAA
GCCGCACGTCTACCTAGTACCCTCGCAGACGGACAGCGCCAGGGAGCAATGGCAGCGCGCCGACCCTGGAT
GGGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGCCGGAAGGGGCGGTGCGG
GAGGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCTGCCCCGCGGGTGTTCGCATTCTGCAAGCCTC
CGGAGCGCACGTGGCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAGGGTtcgaaCACCAT
GTCAAGGCTGGACAAGAGCAAAGTCATAAACTCTGCTCTGGAATTACTCAATGGAGTCGGTATCGAAGGCCT
GACGACAAGGAAACTCGCTCAAAGCTGGGAGTTGAGCAGCCTACCCTGTACTGGCAGGTGAAGAACAAG
CGGGCCCTGCTCGATGCCCTGCCAATCGAGATGCTGGACAGGCATCATACCACTCTGCCCCCTGGAAGGC
GAGTCATGGCAAGACTTTCTGCGGAACAACGCCAAGTCATACCGCTGTGCTCTCTCTCACATCGCGACGGG
GCTAAAGTGCATCTCGGCACCCGCCCAACAGAGAAACAGTACGAAACCCTGGAAATCAGCTCGCGTTCCT

GTGTCAGCAAGGCTTCTCCCTGGAGAACGCACTGTACGCTCTGTCCGCCGTGGGCCACTTTACACTGGGCT
 GCGTATTGGAGGAACAGGAGCATCAAGTAGCAAAAGAGGAAAGAGAGACACCTACCACCGATTCTATGCCC
 CCACTTCTGAAACAAGCAATTGAGCTGTTTCGACCGGCAGGGAGCCGAACCTGCCTTCTTTTCGGCCTGGA
 ACTAATCATATGTGGCCTGGAGAAACAGCTAAAGTGCGAAAGCGGCGGGCCGACCGACGCCCTTGACGATT
 TTGACTTAGACATGCTCCCAGCCGATGCCCTTGACGACTTTGACCTTGATATGCTGCCTGCTGACGCTCTTGA
 CGATTTTGACCTTGACATGCTCcccgggAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGCGACGT
 GGAGGAGAACCCTGGACCTggatccATGACCGAGTACAAGCCCACGGTGCCTCGCCACCCGCGACGACGT
 CCCCAGGGCCGTACGCACCCTCGCCGCCGCGTTTCGCCGACTACCCCGCCACGCGCCACACCGTCGATCCGG
 ACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCTCACGCGCGTCGGGCTCGACATCGGCAA
 GGTGTGGGTCGCGGACGACGCGCGCCCGTGGCGGTCTGGACCACGCCGGAGAGCGTCGAAGCGGGGG
 CGGTGTTCCGCCGAGATCGGCCCGCATGGCCGAGTTGAGCGGTTCCCGGCTGGCCGCGCAGCAACAGAT
 GGAAGGCCTCCTGGCGCCGCACCGGCCCAAGGAGCCCGCGTGGTTCTGGCCACCGTCGGCGTGTGCCCC
 GACCACCAGGGCAAGGGTCTGGGCAGCGCGTCGTGCTCCCCGAGTGGAGGCGGCCGAGCGCGCCGG
 GGTGCCCCCCTTCTGGAGACCTCCGCGCCCCGCAACCTCCCCTTCTACGAGCGGCTCGGCTTACCGTCAC
 CGCCGACGTCGAGGTGCCGAAGGACCGCGCACCTGGTGCATGACCCGCAAGCCCGGTGCCTGAactagt

Table 2. Information of sgRNA target sequences

Target Gene	Target Transcript	Strand	sgRNA Target Sequence (5'-3')	PAM Sequence	Exon Number
NFAT5	NM_138713.3	antisense	ATATGTTGGTCATGATAGGG	AGG	10
		antisense	TGGTATGAGTGGAGTCATCA	GGG	12
		sense	CAAGTCAGTCAAATTCAGAG	TGG	13
		sense	GAGTGCATTGTCTACCAATG	AGG	13
RFP	KT358727.1	sense	CCACAGACTGGAAAGAATCA	AGG	

DNA sequencing of *nfat5* PCR products

Note: sgRNA targeting sequences are underlined. PAM sequences are highlighted in bold italic. The predicted cutting sites of Cas9 are marked in red. The premature termination codon TGA in #11 *nfat5* exon 13 was marked in bold, red font.

(1) HEK-293A *nfat5* exon 13

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TTTTTCAACAAGTCAGTCAAATTCAGAGTGGTGTAAGCCCTGGAATGTTTTCTCAACAGAGCCAACAGTCCATAC
 CAGACCAGATAATTTATTACCTGGAAGAGCTGAAAGTGTTTCATCCACAGTCTGAAAACACGTTATCTAATCAACAG
 CAGCAGCAGCAGCAGCAACAGCAAGTGATGGAATCTTCAGCCGCAATGGTGATGGAGATGCAACAGAGTATCTG
 CCAGGCAGCTGCCAGATTAGTCAGAGTTATTCCTTCAACTGCTTCAGCAAATGGAAACCTTCAGCAATCGCCA
 GTTTACCAGCAGACTTCTCACATGATGAGTGCATTGTCTACCAATGAGGATATGCAAATGCAGTGTGAATTG...

(2) NC *nfat5* exon 13

...

TTTTTCAACAAGTCAGTCAAATTCAGAGTGGTGTAAGCCCTGGAATGTTTTCTCAACAGAGCCAACAGTCCATAC

CAGACCAGATAATTTATTACCTGGAAGAGCTGAAAGTGTTTCATCCACAGTCTGAAAACACGTTATCTAATCAACAG
CAGCAGCAGCAGCAGCAACAGCAAGTGATGGAATCTTCAGCCGCAATGGTGATGGAGATGCAACAGAGTATCTG
CCAGGCAGCTGCCCAGATTCAAGTCAGAGTTATTCCCTTCAACTGCTTCAGCAAATGGAAACCTTCAGCAATCGCCA
GTTTACCAGCAGACTTCTCACATGATGAGTGCATTGTCTACCA**ATG****AGG**ATATGCAAATGCAGTGTGAATTG...

(3) #11 *nfat5* exon 13

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TTTTTCAACAAGTCAGTCAAATTCA**G**GTAGACAATGCACTCATCATG**TGA**GAAAGTCTGCTGGTAAACTGGCGATTG
CTGAAGGTTTCCATTTGCTGAAGCAGTTGAAGGGAATAACTCTGACTGAATCTGGGCAGCTGCCTGGCAGATACT
CTGTTGCATCTCCATCACCATTGCGGCTGAAGATTCCATCACTTGCTGTTGCTGCTGCTGCTGCTGCTGTTGATTAG
ATAACGTGTTTTCAGACTGTGGATGAACACTTTCAGCTCTTCAGGTAATAAATTATCTGGTCTGGTATGGACTGTT
GGCTCTATTGAGGAAAACATTCCAGGGCTTACACCACTCT**ATG****AGG**ATATGCAAATGCAGTGTGAATTG...