
Supporting information

Analysis and Identification of Tumorigenic Targets of MicroRNA in Cancer Cells by
Photo-reactive Chemical Probes

Zhiyu Su, Tsogzolmaa Ganbold, Huricha Baigude*

School of Chemistry & Chemical Engineering, Inner Mongolia Key Laboratory
of Mongolian Medicinal Chemistry, Inner Mongolia University, 235 West
College Road, Hohhot, Inner Mongolia, 010020, PR China

*Correspondence to:

Dr. Huricha Baigude, School of Chemistry & Chemical Engineering, Inner Mongolia
University, 235 West College Road, Hohhot, Inner Mongolia Autonomous Region,
010020 P.R.China, Fax: +86-471-4993165, e-mail: hbaigude@imu.edu.cn

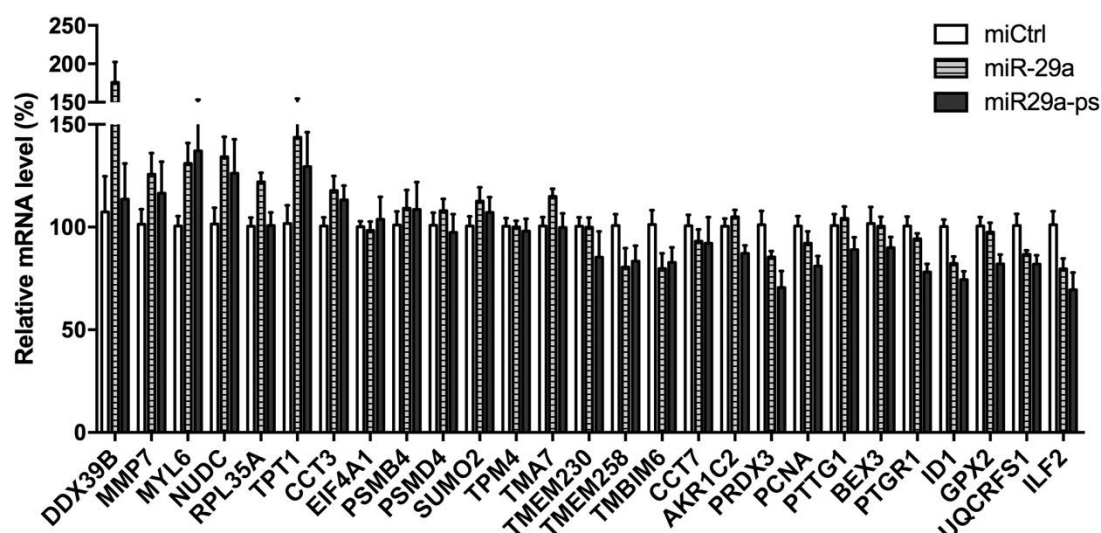


Figure S1. QPCR validation of RNA-Seq result. 27 genes that gave relatively higher reads (FPKM>100) were chosen for QPCR validation. Gene expression in miR-29a or miR-29a-ps treated cells was compared to the control (miCtrl treated cells). *GAPDH* was served as internal control. The average of three independent experiments was expressed as the mean $\pm$ SEM relative to control.

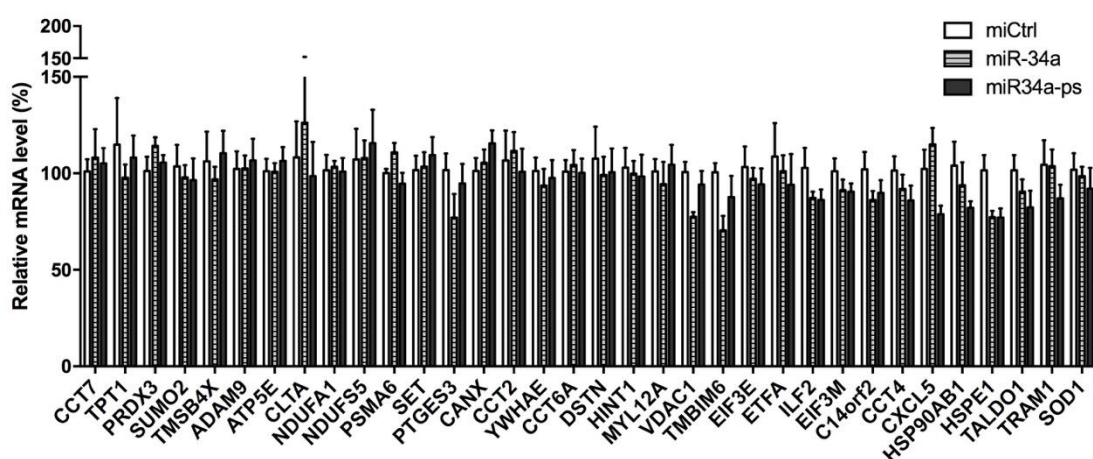


Figure S2. QPCR validation of RNA-Seq result. 34 genes that gave relatively higher reads (FPKM>100) were chosen for QPCR validation. Gene expression in miR-34a or miR-34a-ps treated cells was compared to the control (miCtrl treated cells). *GAPDH*

was served as internal control. The average of three independent experiments was expressed as the mean $\pm$ SEM relative to control.

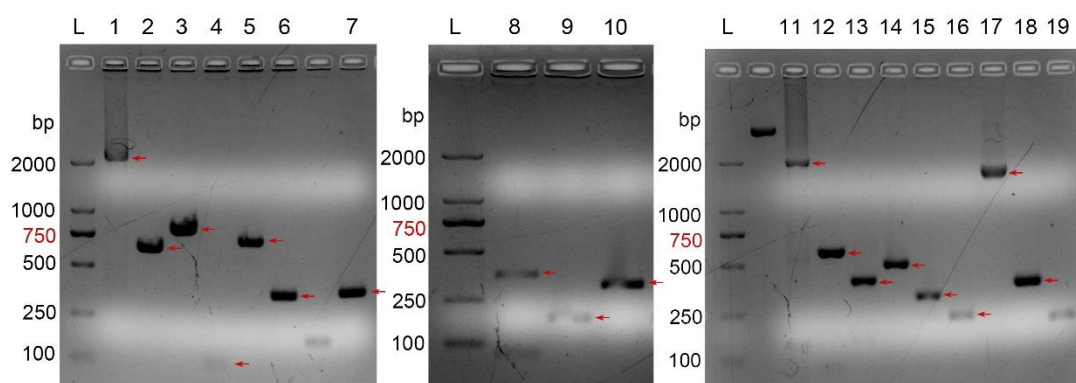


Figure S3. Cloning of 3' UTR of candidate targets genes and construction of luciferase expressing vectors. Agarose gel electrophoresis of 3' UTR fragments of candidate targets of miR-29a and miR-34a. cDNA was yielded from poly(A) RNA extracted from A549 cells, and 3' UTR of candidate genes were cloned by polymerase chain reaction (PCR), respectively, followed by PCR amplification. The PCR products were analyzed on agarose gel electrophoresis. L represents DNA ladder. Lanes 1-19, *AKR1C2* 3' UTR (NM_001321027.1; NM_001354.5; NM_205845.2, 2236 bp), *ILF2* 3' UTR (NM_001267809.1; NM_004515.3, 655 bp), *PRDX3* 3' UTR (NM_001302272.1, 803 bp), *PTTG1* 3' UTR (NM_001282382.1; NM_001282383.1; NM_004219.3, 81 bp), *ID1* 3' UTR (NM_181353.2, 684 bp), *PCNA* 3' UTR (NM_002592.2; NM_182649.1, 330 bp), *GPX2* 3' UTR (NM_002083.3, 353 bp), *BEX3* 3' UTR (NM_001282674.1; NM_014380.2; NM_206915.2; NM_206917.2, 337 bp), *PTGR1* 3' UTR (NM_001146108.1; NM_012212.3, 156 bp), *UQCERS1* 3' UTR (NM_006003.2, 288 bp), *CXCL5* 3' UTR (NM_002994.4, 2012 bp), *CCT4* 3'

UTR (NM_001256721.1; NM_006430.3, 568 bp), *C14orf2* 3' UTR (NM_001127393.1; NM_004894.2, 399 bp), *EIF3M* 3' UTR (NM_001307929.1; NM_006360.5, 474 bp), *HSP90AB1* 3' UTR (NM_001271969.1; NM_001271970.1; NM_001271971.1; NM_001271972.1; NM_007355.3, 290 bp), *HSPE1* 3' UTR (NM_002157.2, 216 bp), *TRAM1* 3' UTR (NM_001317804.1; NM_001317805.1; NM_014294.5, 1714 bp), *SOD1* 3' UTR (NM_000454.4, 368 bp) and *TALDO1* 3' UTR (NM_006755.1, 178 bp) respectively. All of 3' UTR sequences of candidate target have been subjected to sequencing analysis and confirmed precisely.

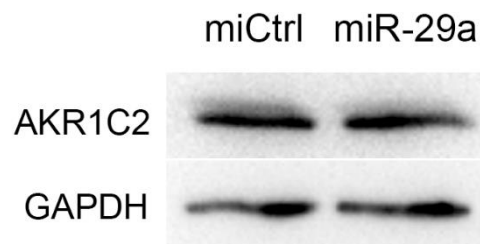


Figure S4. Western blotting analysis showed that the protein level of AKR1C2 did not decrease in miR-29a transfected cells, indicating that it may be a false positive.

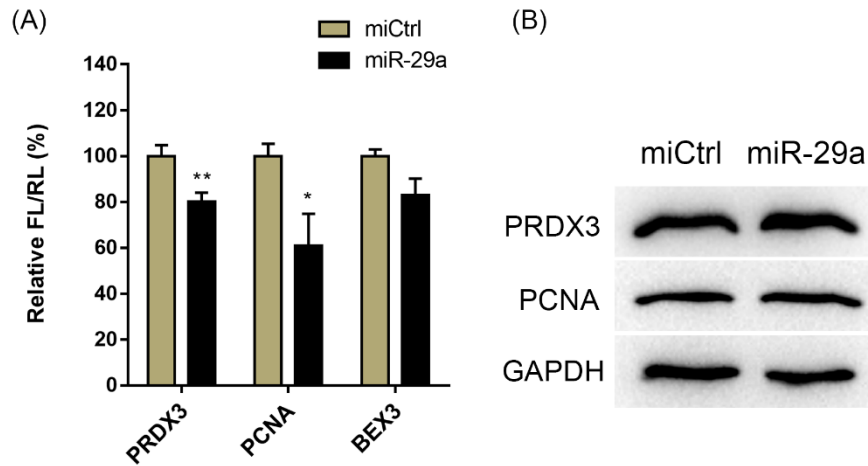


Figure S5. Validation of potential targets (*PRDX3*, *PCNA* and *BEX3*) of miR-29a. (A) Dual luciferase assay showed that *PRDX3*, *PCNA* and *BEX3* do interact with miR-29a in the 3' UTR region of corresponding mRNA. (B) *PRDX3* and *PCNA* protein levels were not decreased in cells transfected with miR-29a, demonstrating that they are false positive. *BEX3* was not to be confirmed due to the lack of efficient antibody against this protein.

Supplementary Table S1. FPKM value of established targets in RNA-Seq.

Symbol	Means-miCtrl	Means-miR-29a	Means-miR-29a-ps
<i>DKK1</i>	235.1366667	176.68	165.76
<i>MCL1</i>	60.51666667	35.41666667	46.37666667
<i>DNMT3A</i>	6.266666667	4.02	4.713333333
Symbol	Means-miCtrl	Means-miR-34a	Means-miR-34a-ps
<i>CDK4</i>	86.17666667	51.79	79.91666667
<i>MET</i>	60.86666667	30.31333333	59.55666667
<i>CCNE2</i>	4.526666667	2.526666667	2.703333333

Supplementary Table S2. List of primers used for qRT-PCR.

Name	Sequence
hsaACTB_F	5'-ccaaccgcgagaagatga-3'
hsaACTB_R	5'-ccagaggcgtagcaggatag-3'
hsaDKK1_F	5'-caggcgtgcaaatctgtct-3'
hsaDKK1_R	5'-aatgattttgatcagaagacacata-3'
hsaMCL1_F	5'-aagccaatgggcagggtct-3'
hsaMCL1_R	5'-tgtccagttccgaagcat-3'
hsaDNMT3A_F	5'-actacatcagcaagcgcaag-3'
hsaDNMT3A_R	5'-cacagcattcattcctgcaa-3'
hsaCDK4_F	5'-gtgcagtcggtggtacctg-3'
hsaCDK4_R	5'-aggcagagattcgcttgtgt-3'
hsaMET_F	5'-tgaaattcatccaacaaatctt-3'
hsaMET_R	5'-aatagaaaactgacaatgttgagagg-3'
hsaCCNE2_F	5'-gccattgattcattagagttcca-3'
hsaCCNE2_R	5'-ctgtcccactccaaacctg-3'
hsaGAPDH_F	5'-agccacatcgctcagacac-3'
hsaGAPDH_R	5'-gccaatacgaccaaattcc-3'
hsaAKR1C2_F	5'-gggtgtccaactcaacca-3'
hsaAKR1C2_R	5'-aagtaaggatgacattccacctg-3'
hsaCCT3_F	5'-gacagacaataatcgattgct-3'
hsaCCT3_R	5'-tgctcctgttccaacatcat-3'
hsaCCT7_F	5'-tgaataagcttccaaatgatgc-3'

hsaCCT7_R	5'-ggccttactctcaactgctgtact-3'
hsaDDX39B_F	5'-tccctgagacttcctctc-3'
hsaDDX39B_R	5'-gcccggtagtgaaggtgac-3'
hsaEIF4A1_F	5'-cgtggattcaaggaccagat-3'
hsaEIF4A1_R	5'-ggtcacctcaagcacatcag-3'
hsaGPX2_F	5'-gtcctggcttccttgc-3'
hsaGPX2_R	5'-tggtcaggatctcctattctg-3'
hsaID1_F	5'-ccagaaccgcaaggtgag-3'
hsaID1_R	5'-ggtcctgatgtagtcgatga-3'
hsaILF2_F	5'-cgcctcttcagttgtctgc-3'
hsaILF2_R	5'-gaccacggcctctgtcac-3'
hsaMMP7_F	5'-cggatggtagcagtctaggg-3'
hsaMMP7_R	5'-aggttgatacatcactgcattag-3'
hsaMYL6_F	5'-tattctgtcggggtagc-3'
hsaMYL6_R	5'-acggactctggggagactg-3'
hsaNUDC_F	5'-gatggggagctctacaatgaa-3'
hsaNUDC_R	5'-ggctccaccactccatctta-3'
hsaPCNA_F	5'-tggagaacttggaatggaaac-3'
hsaPCNA_R	5'-gaactgggtcattcatctctatgg-3'
hsaPRDX3_F	5'-cccgagtgcactgaagatg-3'
hsaPRDX3_R	5'-ccaaggaatggcactcacat-3'
hsaPSMB4_F	5'-ttatggtgcatacttggtca-3'
hsaPSMB4_R	5'-ggcctcgggtctggcttag-3'
hsaPSMD4_F	5'-ttggaggagttgtttaggc-3'

hsaPSMD4_R	5'-cgcatatactcactgtgtgccac-3'
hsaPTTG1_F	5'-ttgacgaggagagagagcttg-3'
hsaPTTG1_R	5'-tgcttgaaggagactgcaac-3'
hsaRPL35A_F	5'-gtttgttctgtgccttgg-3'
hsaRPL35A_R	5'-cagccttcagacatagttcct-3'
hsaSUMO2_F	5'-ggcaaccaatcaatgaaaca-3'
hsaSUMO2_R	5'-agaagcaggttccctttcag-3'
hsaTMBIM6_F	5'-aggcgggaagtgagagga-3'
hsaTMBIM6_R	5'-gtccgtgcagcagtctcc-3'
hsaTPM4_F	5'-gaagagcaaagtctcattcca-3'
hsaTPM4_R	5'-tttgatatggctgacctga-3'
hsaTPT1_F	5'-aggtgctcccactgcactt-3'
hsaTPT1_R	5'-caggctggcctctaacaatc-3'
hsaBEX3_F	5'-cttcggtgcagtcgtcact-3'
hsaBEX3_R	5'-acacttagcctcgagacct-3'
hsaPTGR1_F	5'-gcagccaaaagattgaagga-3'
hsaPTGR1_R	5'-ggctacattttactttccacaact-3'
hsaTMA7_F	5'-ggctttcaagcagaaacaaaa-3'
hsaTMA7_R	5'-caggcacaaggaacagcttac-3'
hsaTMEM230_F	5'-attctgctcatgattccaagg-3'
hsaTMEM230_R	5'-aaactaaggtgatctagtccttaggc-3'
hsaTMEM258_F	5'-cctcactcttcatgggcttt-3'
hsaTMEM258_R	5'-tcagtgaagccatctggttg-3'
hsaUQCRFS1_F	5'-cctgtgttgacctgaagc-3'

hsaUQCRFS1_R	5'-ataacaaacagaagcagggacat-3'
hsaTMSB4X_F	5'-cctgcctttcccatctgtc-3'
hsaTMSB4X_R	5'-cttccaccccacttcttct-3'
hsaADAM9_F	5'-tcccccaaattgtgagactaa-3'
hsaADAM9_R	5'-tccgtccctcaatgcagtat-3'
hsaATP5E_F	5'-agctccgctttcgctaca-3'
hsaATP5E_R	5'-ctctcactgctttgcacaga-3'
hsaC14orf2_F	5'-aactcgcgggtcagacat-3'
hsaC14orf2_R	5'-tggtgtagtagggcttcatgg-3'
hsaCANX_F	5'-atggggcctgaagaaagc-3'
hsaCANX_R	5'-atcatctgccccacaacg-3'
hsaCCT2_F	5'-tgaagcgacaggttcttctg-3'
hsaCCT2_R	5'-gtggtgatcagggacacgtt-3'
hsaCCT4_F	5'-aaatgatggtgctaccattctga-3'
hsaCCT4_R	5'-gccttagacagctccaccag-3'
hsaCCT6A_F	5'-atgtgctgcttcacgaaatg-3'
hsaCCT6A_R	5'-cattagaagtcgtaccatcacca-3'
hsaCLTA_F	5'-caagacaggacgagcagcta-3'
hsaCLTA_R	5'-gtcagcgaagggtgtttgt-3'
hsaCXCL5_F	5'-ggtccttcgagctccttgt-3'
hsaCXCL5_R	5'-gcagctcttcaacacagca-3'
hsaDSTN_F	5'-cctggcatcttggaatcat-3'
hsaDSTN_R	5'-acaatgtagccctaattgcatt-3'
hsaEIF3E_F	5'-ttccagtacgaatgtgggaat-3'

hsaEIF3E_R	5'-ccccagagtgaacttaaagca-3'
hsaEIF3M_F	5'-gagtggagtgccgctgtg-3'
hsaEIF3M_R	5'-ccttagatttcagataagcacga-3'
hsaETFA_F	5'-aatatctggagccatccaaca-3'
hsaETFA_R	5'-tgggtctttattaattgccaca-3'
hsaHINT1_F	5'-cacattttctggtgataccaag-3'
hsaHINT1_R	5'-tgccaacaatcattaagtgtcc-3'
hsaHSP90AB1_F	5'-cggtgctcactattacgtataatcct-3'
hsaHSP90AB1_R	5'-ctgcctgaaaggcaaaagtc-3'
hsaHSPE1_F	5'-ggcgcctagaaaagcctaga-3'
hsaHSPE1_R	5'-acgctgacgcgaagactc-3'
hsaMYL12A_F	5'-ctcacctcgcctgacttaaaa-3'
hsaMYL12A_R	5'-caaacacattggatgttgac-3'
hsaNDUFA1_F	5'-ggtcaccttcaaggacca-3'
hsaNDUFA1_R	5'-gtgaacctgtggatgtacgc-3'
hsaNDUFS5_F	5'-gatttcgtagagtgttgcttcg-3'
hsaNDUFS5_R	5'-gaggtggaggggtgtacttc-3'
hsaPSMA6_F	5'-gctccagagccgtgagtt-3'
hsaPSMA6_R	5'-gttgacgcgactgcttcc-3'
hsaPTGES3_F	5'-gagagaagtcgactccctagca-3'
hsaPTGES3_R	5'-ccctcttctcttctgaatgg-3'
hsaSET_F	5'-aacattgtcaaccatccacaa-3'
hsaSET_R	5'-tctgtcacttcaactctggtcaa-3'
hsaSOD1_F	5'-gcatcatcaatttcgagcag-3'

hsaSOD1_R	5'-caggccttcagtcagtcctt-3'
hsaTALDO1_F	5'-aacctgggaaggaattcagg-3'
hsaTALDO1_R	5'-acgtcatgttgagtgatg-3'
hsaTRAM1_F	5'-tgaactctactccagaaaaccaa-3'
hsaTRAM1_R	5'-cctagatgattcaagttcaaaaggta-3'
hsaVDAC1_F	5'-caggctcctgtgtctgctg-3'
hsaVDAC1_R	5'-gaagacatccctggcagatt-3'
hsaYWHAE_F	5'-agcaggctgagcgatacg-3'
hsaYWHAE_R	5'-tccacatccatccctgctac-3'

Supplementary Table S3. List of primers used for constructing reporter vectors.

Name	Sequence
hsaAKR1C2 3' UTR_F	5'-gccgtgtaattctagacatggagggcattgcatgag-3'
hsaAKR1C2 3' UTR_R	5'-ccgccccgactctagaacccatattgtcacattacaagaagtc-3'
hsaGPX2 3' UTR_F	5'-gccgtgtaattctagatgtgaactgctcaacacacagatc-3'
hsaGPX2 3' UTR_R	5'-ccgccccgactctagaggccgtttccacacctgc-3'
hsaID1 3' UTR_F	5'-gccgtgtaattctagaatcatccttataccgacg-3'
hsaID1 3' UTR_R	5'-ccgccccgactctagataccaccatctaaattatttgc-3'
hsaILF2 3' UTR_F	5'-gccgtgtaattctagacattcccttcactcctttc-3'
hsaILF2 3' UTR_R	5'-ccgccccgactctagacagtgttgatagaatcattttatc-3'
hsaPCNA 3' UTR_F	5'-gccgtgtaattctagagcattctaaaattcaagaaaat-3'
hsaPCNA 3' UTR_R	5'-ccgccccgactctagagatgttgaaattcaagtaact-3'
hsaPRDX3 3' UTR_F	5'-gccgtgtaattctagaatcagtagatcacccatgtg-3'
hsaPRDX3 3' UTR_R	5'-ccgccccgactctagaactgggtggtaaaatgcaaagt-3'
hsaPTTG1 3' UTR_F	5'-gccgtgtaattctagaatttcttagtgcttcagagtt-3'
hsaPTTG1 3' UTR_R	5'-ccgccccgactctagattaagaatctgttaaagaatgct-3'
hsaBEX3 3' UTR_F	5'-gccgtgtaattctagatcctgccattatcatgagat-3'
hsaBEX3 3' UTR_R	5'-ccgccccgactctagaaacactctagtgtatttaatctc-3'
hsaPTGR1 3' UTR_F	5'-gccgtgtaattctagaaaagaggacacatggaatc-3'
hsaPTGR1 3' UTR_R	5'-ccgccccgactctagaacataccacttaaatgtattttatt-3'
hsaUQCRFS1 3' UTR_F	5'-gccgtgtaattctagagacttgactcaagtcatag-3'
hsaUQCRFS1 3' UTR_R	5'-ccgccccgactctagacacaagtatctgtacaccag-3'
hsaCXCL5 3' UTR_F	5'-gccgtgtaattctagattaagagaaatgagcacg-3'

hsaCXCL5 3' UTR_R	5'-ccgccccgactctagagaagtttagaaatctttaatg-3'
hsaC14orf2 3' UTR_F	5'-gccgtgtaattctagaccagatttacttgagtagc-3'
hsaC14orf2 3' UTR_R	5'-ccgccccgactctagaagtagcaggatcaagttaat-3'
hsaCCT4 3' UTR_F	5'-gccgtgtaattctagatctggataactgactagc-3'
hsaCCT4 3' UTR_R	5'-ccgccccgactctagaatgtatacacttttattgcag-3'
hsaHSP90AB1 3' UTR_F	5'-gccgtgtaattctagagttaggagttcatagttgg-3'
hsaHSP90AB1 3' UTR_R	5'-ccgccccgactctagaagaactgtataaaaacggcat-3'
hsaHSPE1 3' UTR_F	5'-gccgtgtaattctagaaataagtcactattgaaatgg-3'
hsaHSPE1 3' UTR_R	5'-ccgccccgactctagaaaagattaaccactcatttac-3'
hsaTALDO1 3' UTR_F	5'-gccgtgtaattctagacgcacccctgaggctggac-3'
hsaTALDO1 3' UTR_R	5'-ccgccccgactctagagaaacagcacaggaaaagtgactgc-3'
hsaTRAM1 3' UTR_F	5'-gccgtgtaattctagatgaattataaactaattgattaatg-3'
hsaTRAM1 3' UTR_R	5'-ccgccccgactctagaagtttaatccagtgattcc-3'
hsaEIF3M 3' UTR_F	5'-gccgtgtaattctagagttttatgcttataattttgttc-3'
hsaEIF3M 3' UTR_R	5'-ccgccccgactctagaacaaccagccaaagaaaag-3'
hsaSOD1 3' UTR_F	5'-gccgtgtaattctagacattcccttgatgtagtc-3'
hsaSOD1 3' UTR_R	5'-ccgccccgactctagagaatttgattctttaatagcc-3'

Supplementary Table S4. List of primers used for reverse PCR.

Name	Sequence
pPTTG1_MT (1-14)_F	5'-ccggctcgctgtagccagagtttggtgtattgtatt-3'
pPTTG1_MT (1-14)_R	5'-gctacagcgagccggctagaattacacggcgatc-3'
pPTGR1_MT (103-124)_F	5'-cgctgaggcacatgccttaataaaatacatttaagtg-3'
pPTGR1_MT (103-124)_R	5'-gcatgtgcctcagcgatgagtactatttctaagac-3'
pILF2_MT (34-55)_F	5'-tcattgcgatgttacgctactgggctttacatgg-3'
pILF2_MT (34-55)_R	5'-gtaacatcgcaatgacagtcttcccccttgggt-3'
pILF2_MT (153-171)_F	5'-gctatcgcatcataggtctccatttgtgacct-3'
pILF2_MT (153-171)_R	5'-ctatgatcgcatagctatcaaaaccagtggaatg-3'
pILF2_MT (175-195)_F	5'-cgtcacttccgatactccatctataatggaggataccaac-3'
pILF2_MT (175-195)_R	5'-gtatcggaagtgacgatgggagactggcagctaag-3'