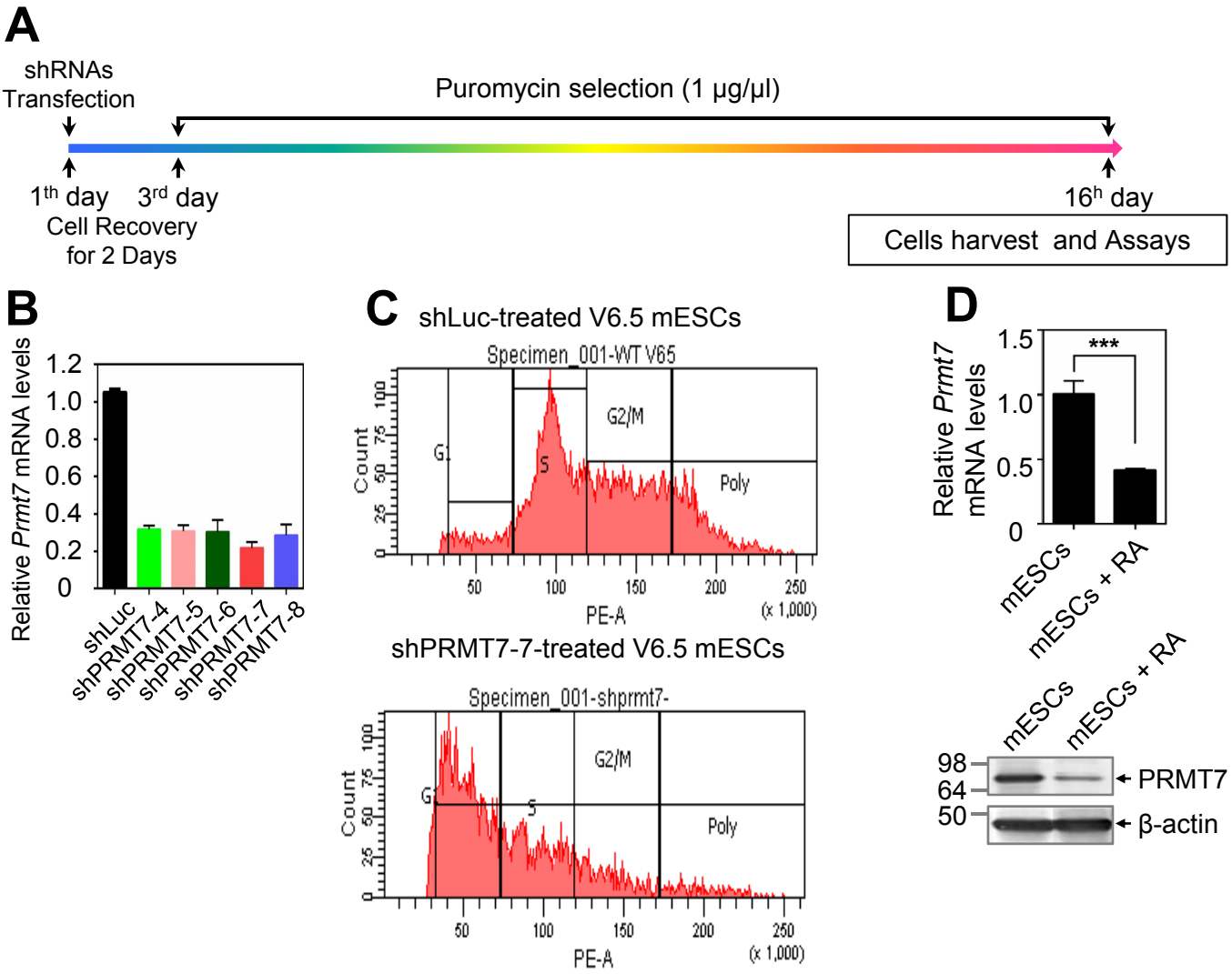
Supplementary Figure S1



Supplementary Figure S1.

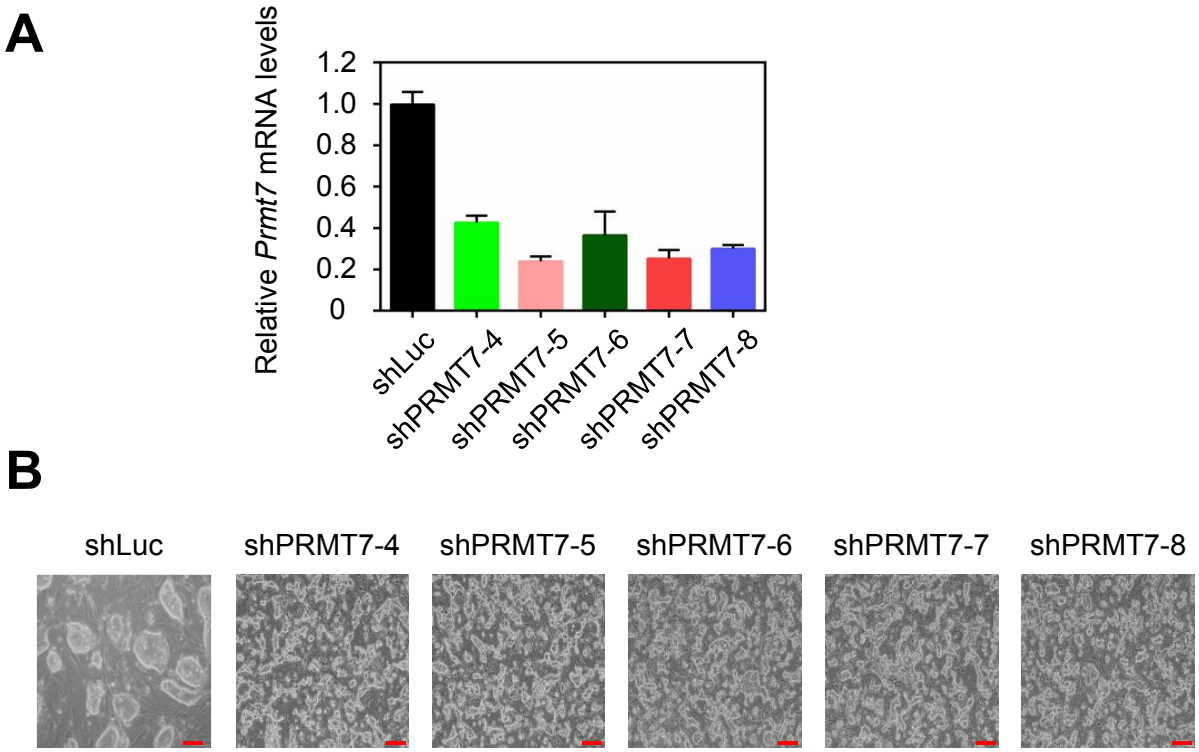
(A) The outline for shRNA treatment and subsequent puromycin selection of V6.5 mESCs.

(**B**) Analysis of *Prmt7* mRNA levels in shLuciferase (shLuc)-treated and shPRMT7-depleted V6.5 mESCs using quantitative RT-PCR.

(**C**) PRMT7 knockdown increases cell population in G1 phase while reducing cell percentages in S and G2/M phases. Cells were electroporated with different shRNAs containing a puromycin-resistant gene, recovered for 2 days, and subjected to puromycin selection for another 10-14 days. Cell cycle analysis indicates spontaneous mESC differentiation. The distribution of shLuc- or shPRMT7-treated V6.5 mESCs in G1, S, and G2/M phase of the cell cycle are shown. Cells were harvested, fixed in cold 75% ethanol for 30 min at 4°C, and washed 2 times with PBS. Then, cells were treated with a buffer containing 50 μg/ml propidium iodine, 5 mM $MgCl_2$, 10 mM TRIS-HCl pH 7.0, 25 μg/ml RNaseA at 37°C for 30 min. DNA contents were examined using flow cytometry.

(**D**) Quantitative RT-PCR and Western analyses showed that PRMT7 mRNA and protein levels were decreased in differentiated V6.5 mESCs that were generated by RA treatment of embryoid body (EB).

Supplementary Figure S2

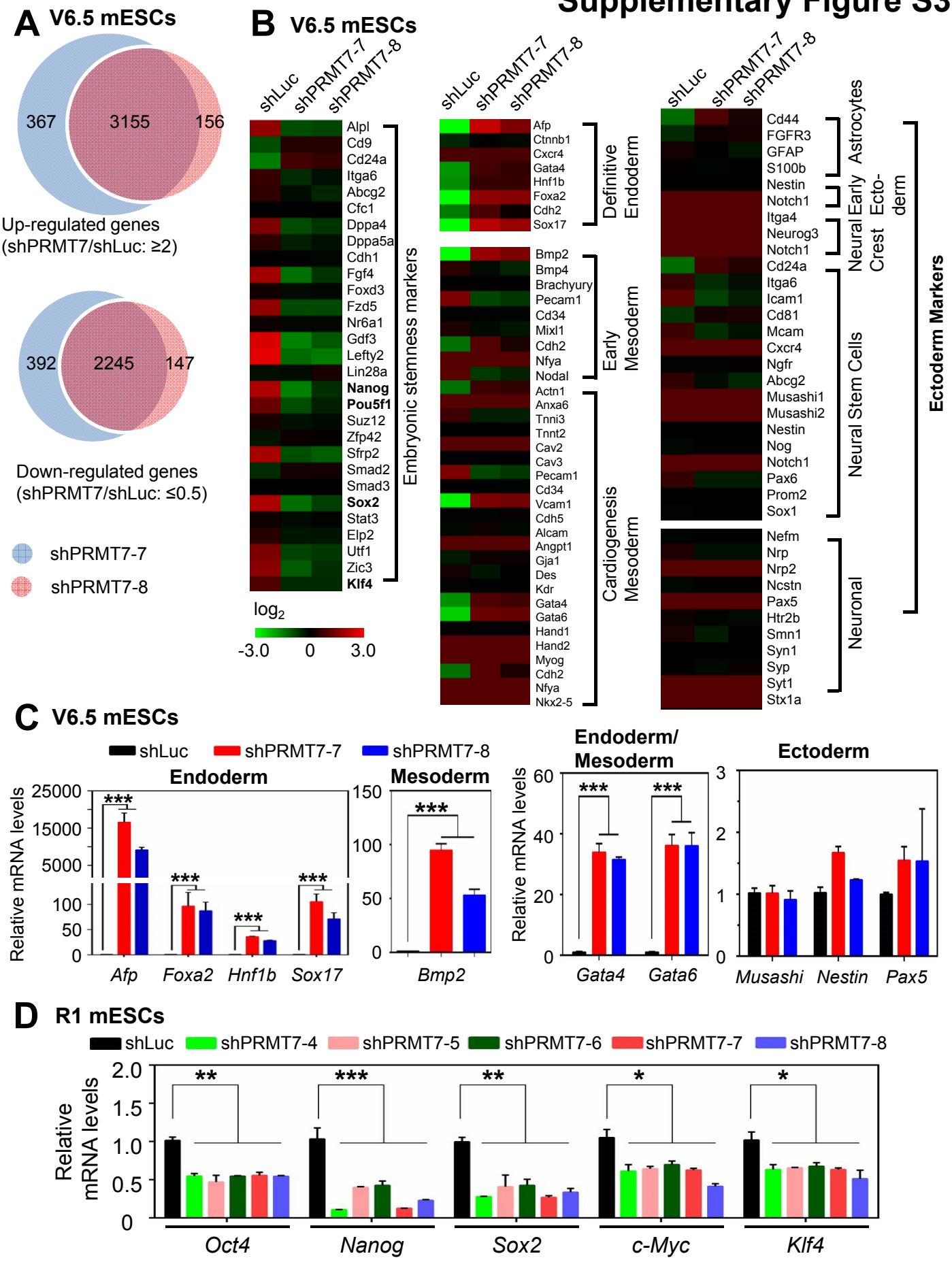


<u>Supplementary Figure S2</u>. PRMT7 depletion results in spontaneous differentiation of R1 mESCs.

(A) Quantitative RT-PCR analysis of *Prmt7* mRNA levels in shLuc- and shPRMT7 (shPRMT7-4 to -8)-treated R1 mESCs.

(**B**) Microscopic images demonstrated that shPRMT7-treated R1 mESCs were differentiated. Red scale bar, 100 μm.

Supplementary Figure S3



<u>Supplementary Figure S3</u>. PRMT7 knockdown downregulated several pluripotent genes and upregulated multiple mesoderm- and endoderm-specific genes.

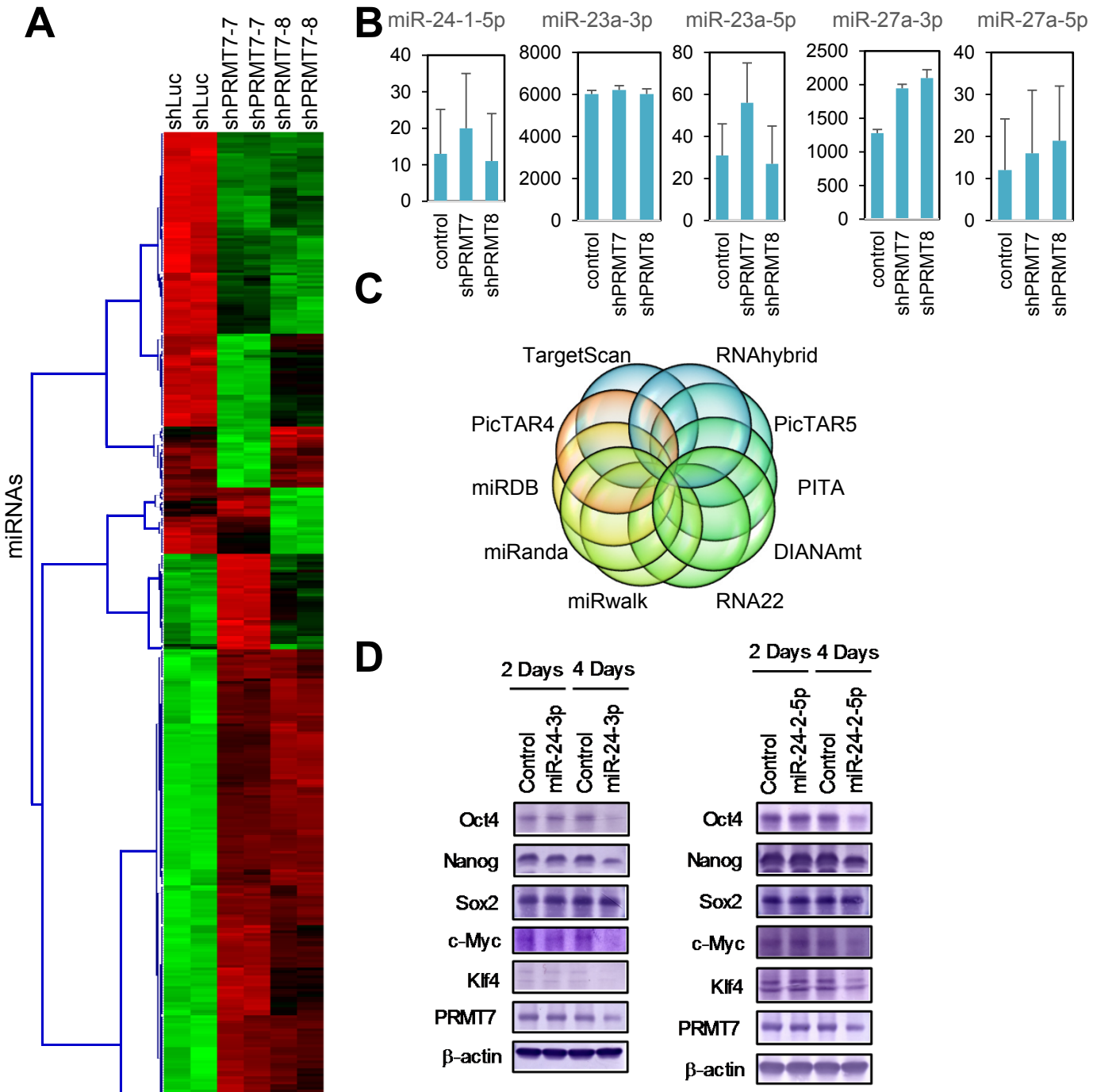
(**A**) Venn diagrams of genes that are upregulated or downregulated at least 2 fold by shPRMT7s (shPRMT7-7 or shPRMT7-8) in V6.5 mESCs. Genes (3155/3522 = 89.6%) upregulated by shPRMT7-7 highly overlap those (3155/3311 = 95.3%) upregulated by shPRMT7-8. Similarly, genes (2245/2637 = 85.1%) downregulated by shPRMT7-7 highly overlap those (2245/2392 = 93.9%) downregulated by shPRMT7-8.

(**B**) Heat maps of stemness, endoderm, mesoderm, and ectoderm marker genes that are compared between shLuc-treated and PRMT7-depleted V6.5 mESCs.

(**C**) Comparison of mRNA levels of several endoderm-, mesoderm- or ectoderm-associated genes between shLuc-treated and PRMT7-depleted V6.5 mESCs using quantitative RT-PCR analysis.

(**D**) Quantitative RT-PCR analysis showed that *Oct4*, *Nanog*, *Sox2, c-Myc,* and *Klf4* mRNA levels were decreased in shPRMT7-treated R1 mESCs compared to those in shLuc-treated cells.

Supplementary Figure S4



Supplementary Figure S4.

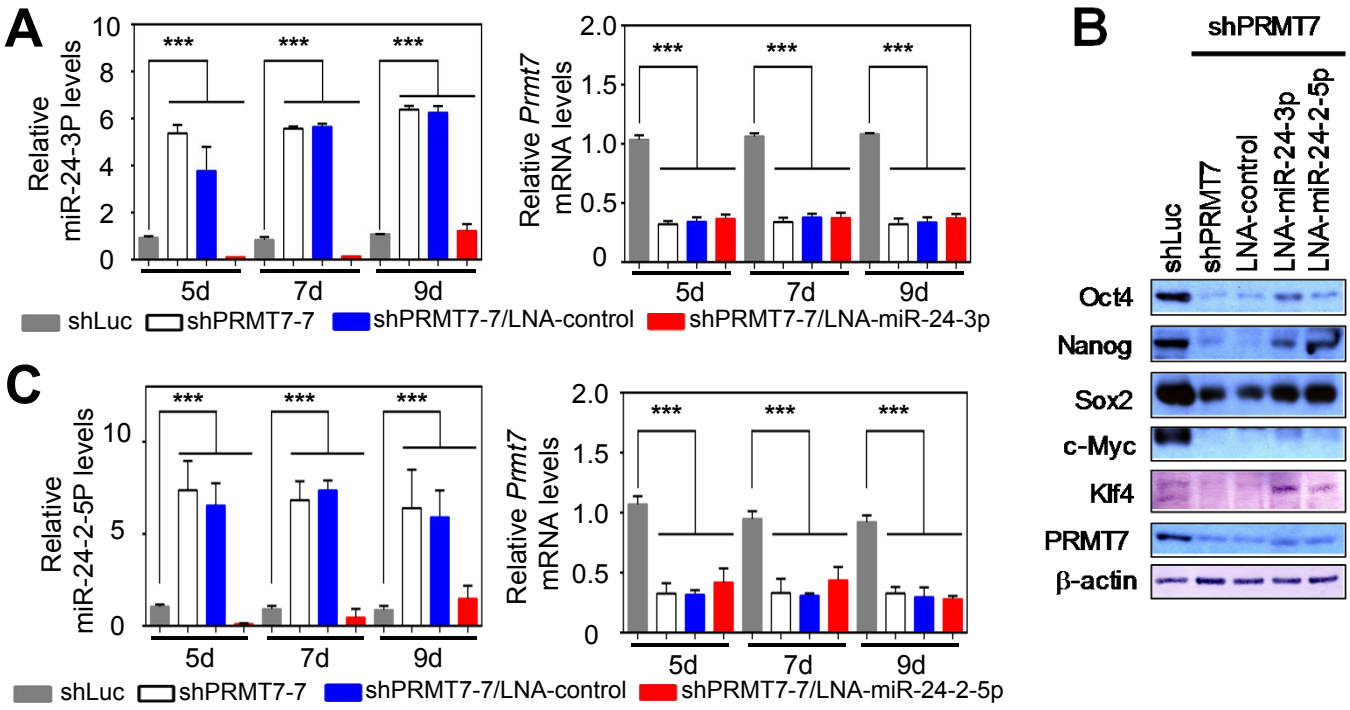
(**A**) A heat map for the whole miRNA list that were analyzed by a miRNA array. Mouse ESCs were treated by shLuc, shPRMT7-7- or shPRMT7-8.

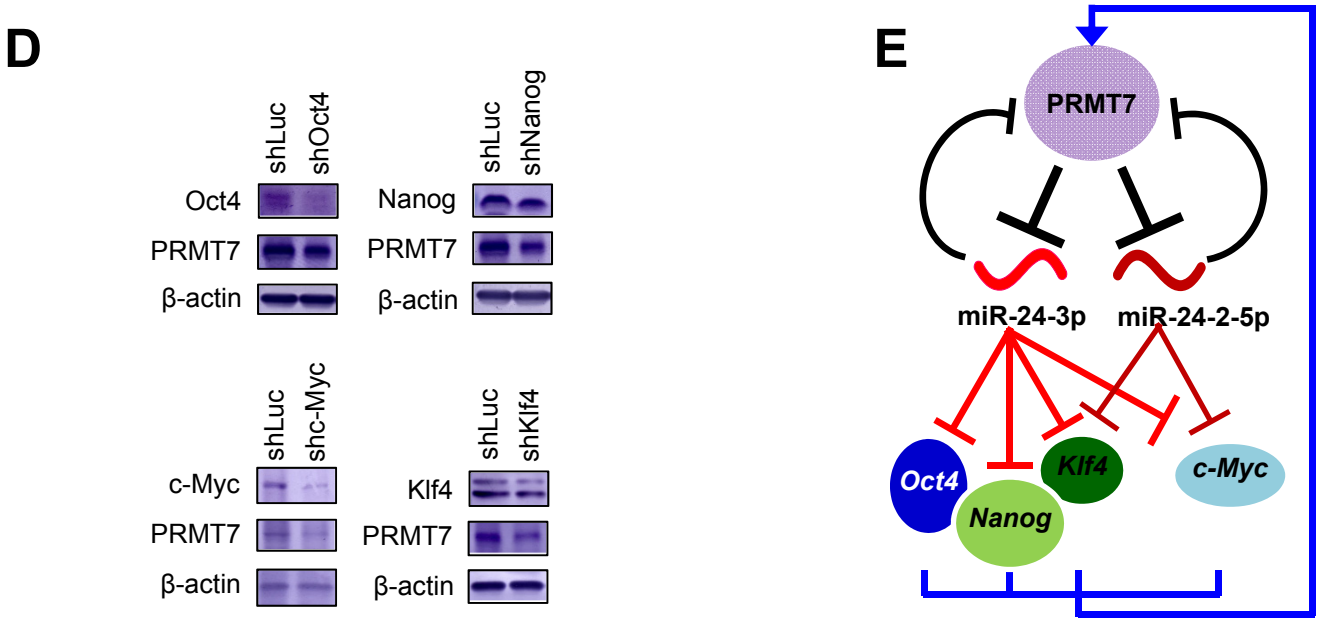
(**B**) miRNA microarray analysis showed that miR-24-1-5p, miR-23a-3p, miR-23a-5p, miR-27a-3p and miR-27a-5p levels were not substantially changed by PRMT7 knockdown. miR-23a and miR-27a are localized at miR24-2 locus. miR-24-3p is expressed from both miR-24-1 and miR-24-2 locus.

(**C**) A diagram showing 10 different prediction programs for miRNA target sites.

(**D**) Western blot analysis showed that protein levels of Oct4, Nanog, Sox2, c-Myc, and Klf4 were reduced in V6.5 mESCs treated with miR-24-3p and miR-24-2-5p mimics (Related to **Figure 4C–F**) .

Supplementary Figure S5





Supplementary Figure S5.

(**A and C**) miR-24-3p (**A**) and miR-24-2-5p (**B**) levels were increased in shPRMT7-treated V6.5 mESCs as compared to shLuc-treated mESCs (**left panels**). LNA-miR-24-3p (or LNA-miR-24-2-5p) treatment in shPRMT7-treated V6.5 mESCs at the 5th, 7th, or 9th day after shPRMT7 transfection decreased miR-24-3p (or miR-24-2-5p) levels as compared to LNA-control treatment (**left panels**). However, PRMT7 mRNA levels in shPRMT7-treated V6.5 mESCs were not changed by the treatment of LNA-miR-24-3p or LNA-miR-24-2-5p (**right panels**) (Related to Figure 5).

(B) The treatment of LNA-miR-24-3p and LNA-miR-24-2-5p at the 7thday after shPRMT7-7 transfection restored at least in part Oct4, Nanog, Sox2, c-Myc and Klf4 protein levels in RMT7-depleted V6.5 mESCs (Related to Figure 5).

(D) Knockdown of Oct4, Nanog, Sox2, c-Myc and Klf4 decreased PRMT7 proteins levels in V6.5 mESCs (Related to Figure 7).

(E) A proposed model. The feedback regulatory loop consisting of PRMT7 and miR-24-3p/miR-24-2-5p is interactive with the core pluripotent factors Oct4, Nanog, Klf4 and c-Myc to regulate mESC pluripotency.

Supplementary Table S1

Supplementary Table S1. Oligonucleotide sequences.

		5'-3' Forward Sequence	5'-3' Reverse Sequence
Cloning & Mutation	h.PRMT7	CCCGAATTCGCCACCATGAAGATCTTCTGCAGTCG	CCCGCGGCCGCGTCTGGGGTATCTGCATGCCTGAA
	m.PRMT7	CCCGAATTCGCCACCATGAAGGTCTTCTGTGGCCG	CCCGCGGCCGCTTTCTCAATAAGAGATCAGCT
	PRMT7(E144A)	CAACATCCTGGTCACAGcGTTGTTTGACACAGAGC	GCTCTGTGTCAAACAACgCTGTGACCAGGATGTTG
	PRMT7(E153A)	GCTGATCGGGGcGGGGGCGCTGC	GCAGCGCCCCCgCCCCGATCAGC
	PRMT7 (E144/153A)	CAACATCCTGGTCACAGcGTTGTTTGACACAGAGCTGATC GGGGcGGGGGCGCTGC	GCAGCGCCCCCgCCCCGATCAGCTCTGTGTCAAACAACgCTGTGA CCAGGATGTTG
	PRMT7(E478A)	CTCCTCCTGGGCGcGCCGTTCTTCACT	AGTGAAGAACGGCgCGCCCAGGAGGAG
	PRMT7(H644A)	CTGCTGCTGGAACCCCgcCTGCAAGCAGGCCGTC	GACGGCCTGCTTGCAGgcGGGGTTCCAGCAGCAG
	L-*Oct4*	AAAACTAGTGGCACCAGCCCTCCCTGGGGATGCT	AAAAAGCTTGCCAGGACTACACAGAACTCATCTT
	L-m.*Oct4* (Mut-01)	CCCTCCCTGGGGATGCTGaaaaacaAGGCAAGGGAGGTAGA	TCTACCTCCCTTGCCTtgttttCAGCATCCCCAGGGAGGG
	L-m.*Oct4* (Mut-02)	CATGGGATCCCATTACAGATGGTTGaaaaaaaCCATGTGGTTGCT GGGAATTGAACT	AGTTCAATTCCCAGCAACCACATGGttttttCAACCATCTGTAA TGGGATCCCATG
	L-m.*Oct4* (Mut-03)	AAGAGCAGTCAGTGCTCTTAACCGCaaaaaaaTCTCTCCAGCCCT CAAACTCTTTTT	AAAAAGAGTTTGAGGGCTGGAGAGAttttttGCGGTTAAGAGCA CTGACTGCTCTT
	L-m.*Oct4-5p* (Mut-01)	gccctccctggggatgctgtgagccataaatagggaggtagacaa	ttgtctacctccctatttatggctcacagcatccccagggagggc
	L-m.*Oct4-5p* (Mut-02)	tggggttggagcccaacctatagagaataaatttgcatattcgccatc ctagaggcg	cgcctctaggatggcgaatatgcaaatttattctctataggttgggct ccaacccca
	L-*Nanog*	AAAACTAGTGACTTACGCAACATCTGGGCTTAAA	AAAAAGCTTAAACCCTGTCTTGATAAACAAAAAG
	L-m.*Nanog* (Mut-01)	GCGTCAGATCTTGTTACGTATGGTTGaaaaaaaCCATGTGGTTGC TGGGATTTGAACT	AGTTCAAATCCCAGCAACCACATGGttttttCAACCATACGTAA CAAGATCTGACGC
	L-m.*Nanog* (Mut-02)	CTTCGGAAGAGCAGTCGGGTGCTCTTATCCaaaaaaaaaTCTCAC CAGCCCCTGGTTTATTTTTTTAA	TTAAAAAAATAAACCAGGGGCTGGTGAGAtttttttttGGATAAG AGCACCCGACTGCTCTTCCGAAG
	L-m.*Nanog*-5p	caaacctaggacttagaacatgcagaaatacaactcaacttctgagct ct	agagctcagaagttgagttgtatttctgcatgttctaagtcctaggtt tg
	L-*Sox2*	AAAACTAGTTGGACACTGAAATTATGCCTGA	AAAAAGCTTACGTCGCCAGCCTCTTACT
	L-m.*Sox2* (Mut-01)	Cttggggaactggataactgtactaaatccaagatcttgatgttacca gc	gctggtaacatcaagatcttggatttagtacagttatccagttcccca ag
	L-m.*Sox2* (Mut-02)	tgcagctggtacacgcaaaagagataaatggcaacagacatattacag aag	cttctgtaatatgtctgttgccatttatctcttttgcgtgtaccagct gca
	L-m.*Sox2*-5p	cagctggtacacgcaaaagagaagcctaaatacagacatattacagaa ga	tcttctgtaatatgtctgtatttaggcttctcttttgcgtgtaccagc tg
	L-*Klf4*	AAAACTAGTTTTCTAACCTTTCACACTGTCTTCC	AAAAAGCTTGGGGAAGGTTCTTTTGTTACTTAGA
	L-m.*Klf4*	gtggttctaaaggtaccaaacgggtttaataagttctccaactgctg catacttttga	tcaaaagtatgcagcagttggagaacttattaaaccccgtttggtacc tttagaaccac
	L-m.*Klf4*-5p	gaagccaaccttaccttagcaagaggattaattatacttgctttttta gactactcagt	actgagtagtctaaaaaagcaagtataattaatcctcttgctaaggta aggttggcttc
	L-*c-Myc*	AAAACTAGTACGAGAACAGTTGAAACACAAACTC	AAAAAGCTTGCACTAAGGATAAAGTGGTTTTGAA
	L-m.*c-Myc* (Mut-01)	ctggggctttgggactgtaagctacttaattaattttaactgcctcaa acttaaatagt	actatttaagtttgaggcagttaaaattaattaagtagcttacagtcc caaagccccag
	L-m.*c-Myc* (Mut-02)	tccgctggttaggggtctgagtaaaaagctacaggctccattaggag c	gctcctaatggagcctgtagctttttactcagacccctaaccagcgg a
	L-m.*c-Myc-5p* (Mut-01)	gggtggagggtgttgtgtgtactcagttattaatagctcactcctacc ttacccactt	aagtgggtaaggtaggagtgagctattaataactgagtacacacaaca ccctccaccc
	L-m.*c-Myc-5p* (Mut-02)	aaaaccagagctgttagttaggaatgggcaaataattagtgagaaggc tagatgcagg	cctgcatctagccttctcactaattatttgcccattcctaactaacag ctctggtttt
	L-*Prmt7*	AAAACTAGTCAGACACCTTGAGCTGATCTCTT	AAAAAGCTTGGGTTAGGGTAAAATGTTTGGAG
	L-m.*Prmt7* (Mut-01)	ctagaacaagacgccgagctaataaatcctcacgggtttatttttctcc t	aggagaaaataaacccgtgaggatttattagctcggcgtcttgttcta g
	L-m.*Prmt7* (Mut-02)	aaaacagtatttctaaataaataaaagttaatacaatagttaaaaagc	gctttttaactattgtattaacttttatttatttagaaatactgtttt
	L-m.*Prmt7*-5p (Mut-01)	atctgcacagcatcaggtgtgactaaatgcataggctggcgagtgact g	cagtcactcgccagcctatgcatttagtcacacctgatgctgtgcaga t
	L-m.*Prmt7*-5p (Mut-02)	agaggtgaccttgtgataacagtaaataagggcagtacaccttgccta ag	cttaggcaaggtgtactgccctttatttactgttatcacaaggtcacct ct
	miR24-3P mimic	UGGCUCAGUUCAGCAGGAACAG	CUGUUCCUGCUGAACUGAGCCA
	miR24-2-5p mimic	GUGCCUACUGAGCUGAAACAGU	ACUGUUUCAGCUCAGUAGGCAC

Supplementary Table S1. Continued.

		5'-3' Forward Sequence	5'-3' Reverse Sequence
	mGAPDH	CATGGCCTTCCGTGTTCCTA	GCCTGCTTCACCACCTTCTT
	m18s RNA	TAGAGGGACAAGTGGCGTTC	CGCTGAGCCAGTCAGTGT
	mPRMT7	CAGGTAGCGTCTGCCTTTGT	CAACTCAGGCCGTTTATTGATGA
	mNanog	TCTTCCTGGTCCCCACAGTTT	GCAAGAATAGTTCTCGGGATGAA
	mSox2	GCTCGCAGACCTACATGAAC	GCCTCGGACTTGACCACAG
	mOct4	AGAGGATCACCTTGGGGTACA	CGAAGCGACAGATGGTGGTC
	mcMyc	CACCATGCCCCTCAACGTGAACTTCACC	TTATGCACCAGAGTTTCGAAGCTGTTCG
	mKlf4	GTGCCCCGACTAACCGTTG	GTCGTTGAACTCCTCGGTCT
	Luciferase	TCCTCTGACACATAATTCGCC	GCTATTCTGATTACACCCGAGG
	miR-24-3p	TGGCTCAGTTCAGCAGGAACAG	*PerfeCTa Universal PCR Primer
	miR-24-2-5p	GTGCCTACTGAGCTGAAACAGT	*PerfeCTa Universal PCR Primer
	miR-185-5p	TGGAGAGAAAGGCAGTTCCTGA	*PerfeCTa Universal PCR Primer
RT-PCR	miR-186-5p	CAAAGAATTCTCCTTTTGGGCT	*PerfeCTa Universal PCR Primer
	miR-200c-5p	CGTCTTACCCAGCAGTGTTTGG	*PerfeCTa Universal PCR Primer
	miR-221-3p	AGCTACATTGTCTGCTGGGTTTC	*PerfeCTa Universal PCR Primer
	miR-221-5p	ACCTGGCATACAATGTAGATTTCTGT	*PerfeCTa Universal PCR Primer
	miR-431-5p	TGTCTTGCAGGCCGTCATGCA	*PerfeCTa Universal PCR Primer
	miR-466a-3p	TATACATACACGCACACATAAGA	*PerfeCTa Universal PCR Primer
	miR-466b-3p	ATACATACACGCACACATAAGA	*PerfeCTa Universal PCR Primer
	SNORD47	GTGATGATTCTGCCAAATGATACAAAGTGATATCACCTTTAAACCG TTCCATTTTATTTCTGAGG	*PerfeCTa Universal PCR Primer
	SNORD66	GTCAGTGCCACGTGTCTGGGCCACTGAGACCACATGATGGGATTGA GGACCTGAGGAA	*PerfeCTa Universal PCR Primer
	miR-24-2		
	a	TGTCATGTAGGTTCTGGGAAAG	GATCTCTGCACTTGGGAACA
	b	AAGAGCAGTAGCCACTCTTAA C	GTGTAGGGAGTTTCAGACCATC
ChIP	c	GGCCTAAAACTCATCATGTAGC	AGTTGGTGGCTCACGTTTATC
	d	TAGAGGAGGGCTAGGGTGTG	GCTTGCCTGCCTATCTTGAC
	e	GGATGGGATTTGATGCCAGT	CACAGTGGCTAAGTTCCG
	f	CCACGATGCCATGAAGAAAC	GTACCTACCACCTGCTCAATTA
	miR-24-2-0.5kb	AATGCTCGAGTGTGGTGAGGTGTACCTATAATCCT	CAATAAGCTTAGAGCCCTGCCAGCCAGGAGGCAGA
Promoter	miR-24-2-1.0kb	AATGCTCGAGGCATCGGCCTGCTTCTCCTTGCTTG	CAATAAGCTTAGAGCCCTGCCAGCCAGGAGGCAGA
Cloning	miR-24-2-1.5kb	AATGCTCGAGAGGACAACTTGCAAGGGTCCGTATG	CAATAAGCTTAGAGCCCTGCCAGCCAGGAGGCAGA
	miR-24-2-2.0kb	AATGCTCGAGGAAGCTGGAGTTACAGGTAGTTGTG	CAATAAGCTTAGAGCCCTGCCAGCCAGGAGGCAGA

* These RT-PCR primers were from Quanta Biosciences

Supplementary Table S3

Supplementary Table S3. A list for miRNAs that are at least 2 fold up-regulated by PRMT7 knockdown and are predicted to target the 3' UTR of *Oct4*, *Nanog*, and/or *Sox2* by miRNA target prediction programs

Mouse miRNA Name	shPRMT7-7 /shLuc	shPRMT7-8 /shLuc	Target	Predicted by the following programs
miR-24-3p	2.021	2.354	*Oct4, Nanog*	DIANAmT, miRanda, miRWalk, PICTAR5, TargetScan
miR-24-2-5p	3.927	3.637	*Oct4*, *Nanog*	DIANAmT, miRanda, miRWalk, PICTAR5, TargetScan
miR-466a-3p	3.353	3.004	*Sox2*	miRanda, miRWalk, PICTAR5, TargetScan
miR-466b-3p	3.256	2.885	*Sox2*	miRanda, miRWalk, PICTAR5, TargetScan
miR-542-3p	2.385	3.263	*Oct4*	DIANAmT, miRanda, miRWalk, PICTAR5, TargetScan
miR-221-3p	4.033	2.409	*Nanog*	DIANAmT, miRWalk, PICTAR5, PITA
miR-221-5p	2.271	1.862	*Nanog*	DIANAmT, miRWalk, PICTAR5, PITA
miR-431-5p	2.228	2.470	*Sox2*	DIANAmT, miRanda, miRWalk, PICTAR5, TargetScan
miR-186-5p	2.221	3.637	*Oct4*	DIANAmT, miRanda, miRWalk, PICTAR5, TargetScan
miR-200c-5p	2.184	1.694	*Sox2*	DIANAmT, miRanda, miRDB, miRWalk, RNAhybid, PICTAR5, TargetScan

Supplementary Table S4

Supplementary Table S4. Putative target sites of miR-24-3p and miR-24-2-5p (**B**) in the 3'UTRs of *Oct4*, *Nanog*, *Klf4*, *c-Myc*, and *Sox2*.

Target genes	Position	miR-24-3p target sites in the 3'UTR regions
Oct4	12-33	5′ CCUCCCUGGGGAUGCUGUGAGCCA 3′ GACAAGGACGACUUGACUCGGU
	617-638	5′ CCCAUUACAGAUGGUUGUGAGCCA 3′ GACAAGGACGACUUGACUCGGU
	688-707	5′ TCAGUGCUCUUAACCGCUGAGCCA 3′ GACAAGGACGACUUGACUCGGU
Nanog	152-174	5′ UCUUGUUACGUAUGGUUGUGAGCCA 3′ GACAAGGACGAC-UUGACUCGGU
	222-244	5′ UCGGGUGCUCUUAUCCACUGAGCCA 3′ GACAA-GGACGACUUGACUCGGU
c-Myc	129-150	5′ CUUUGGGACUGUAAGCUUCAGCCA 3′ GACAAGGACGACUUGACUCGGU
	1100-1121	5′ GGUUAGGGGUCUGAGAAUGAGCGC 3′ GACAAGGACGACUUGACUCGGU
Klf4	582-603	5′ UAAAGGUACCAAACGGGGGAGCCA 3′ GACAAGGACGACUUGACUCGGU
Sox2	60-81	5′ GGGAACUGGAUAACUGUUCAGCCA 3′ GACAAGGACGACUUGACUCGGU
	763-784	5′ CUGGUACACGCAAAAGAGAAGCCA 3′ GACAAGGACGACUUGACUCGGU

Target genes	Position	miR-24-2-5p target sites in the 3'UTR regions
Oct4	18-39	5' UGGGGAUGCUGUGAGCCAAGGCAA 3' UGACAAAGUCGAGUCAUCCGUG
	371-392	5' AGCCCAACCUAUAGAGGAAGGCAU 3' UGACAAAGUCGAGUCAUCCGUG
Nanog	488-509	5' UAGGACUUAGAACAUGCUGGGCAC 3' UGACAAAGUCGAGUCAUCCGUG
c-Myc	660-681	5' UAGUUAGGAAUGGGCAAAAGGCAA 3' UGACAAAGUCGAGUCAUCCGUG
	1381-1402	5' GGUGUUGUGUGUACUCAGAGGCAC 3' UGACAAAGUCGAGUCAUCCGUG
Klf4	1085-1106	5' ACCUUAGCAAGAGGAUGUAGGCAA 3' UGACAAAGUCGAGUCAUCCGUG
Sox2	768-789	5' ACACGCAAAAGAGAAGCCAGGCAA 3' UGACAAAGUCGAGUCAUCCGUG

Supplementary Table S5

Supplementary Table S5. Putative target sites of miR-24-3p and miR-24-2-5p in the 3'UTR of *Prmt7.*

microRNA	Position	mir24-3p and miR-24-2-5p target sites in the 3'UTR regions of PRMT7
miR-24-3p	885-906	5′ GAACAAGACGCCGAGCUUGAGCCU (UTR) 3' GACAAGGACGACUUGACUCGGU
	1165-1186	5′ AACAGUAUUUCUAAAUCUGAGCAA(UTR) 3' GACAAGGACGACUUGACUCGGU
miR-24-2-5p	117-138	5' CACAGCAUCAGGUGUGGCAGGCAG(UTR) 3' UGACAAAGUCGAGUCAUCCGUG
	1579-1600	5' GUGACCUUGUGAUAACAGAGGCAC(UTR) 3' UGACAAAGUCGAGUCAUCCGUG