

Supplementary Information

Argonaute 2-dependent Regulation of Gene Expression by Single-stranded miRNA Mimics

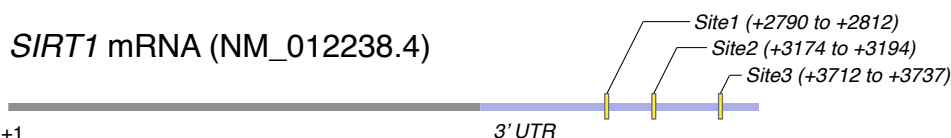
Masayuki Matsui¹, Thazha P. Prakash², and David R. Corey^{1,*}

¹ Departments of Pharmacology and Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390-9041, USA.

² Department of Medicinal Chemistry, Ionis Pharmaceuticals, Carlsbad, CA 92010, USA

* Corresponding author (david.corey@utsouthwestern.edu)

A



Site1: (M)

```
3' ugUUGGU-CGAUUCUGUGACGGu 5' hsa-miR-34a-5p
      ||::| | | | | | | |
5' acAAUUUUUUAAACACUGGCa 3' SIRT1 (+2790 to +2812)
```

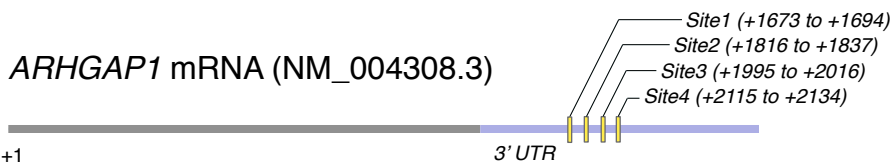
Site2: (M, T)

```
3' uguUGGUCGAUUCUGUGACGGu 5' hsa-miR-34a-5p
      || || | | | | | |
5' uccACAAG-UAUUAAACUGCCa 3' SIRT1 (+3174 to +3194)
```

Site3: (M, T)

```
3' ugUUGGUCGAUUCU----GUGACGGu 5' hsa-miR-34a-5p
      | | | | | | | | | | | |
5' acACCCAGCUAGGACCAUACUGCCa 3' SIRT1 (+3712 to +3737)
```

B



Site1: (M, T)

```
3' uguuggucgauucuGUGACGGu 5' hsa-miR-34a-5p
      | | | | | |
5' ugccuuacacuucuCACUGCCu 3' ARHGAP1 (+1673 to +1694)
```

Site2: (M, T)

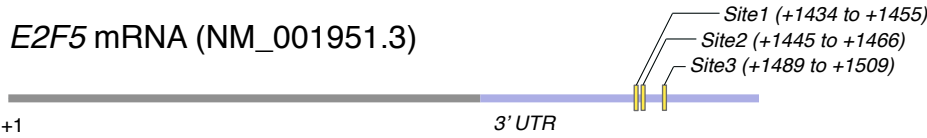
```
3' uguuggucgauucugUGACGGu 5' hsa-miR-34a-5p
      | | | | | |
5' uggaugaaucaaaugACUGCCg 3' ARHGAP1 (+1816 to +1837)
```

Site3: (M, T)

```
3' uguuGGUCGAUUCUGUGACGGu 5' hsa-miR-34a-5p
      | : | | | | : | | | | |
5' caccCUUGCUCUGUACUGCCc 3' ARHGAP1 (+1995 to +2016)
```

Site4: (M, T)

```
3' uguuGGUCGAUUCUGUGACGGu 5' hsa-miR-34a-5p
      | | | | | | | | | |
5' uuucCCAAGU--GAUACUGCCc 3' ARHGAP1 (+2115 to +2134)
```

C**Site1:** (M, T)

```

3' uguuggucgauucuGUGACGGu 5' hsa-miR-34a
      :|||||
5' uugucuuaauuuuuUACUGCCa 3' E2F5 (+1434 to +1455)

```

Site2: (M)

```

3' uguugGUCGAUUCUGUGACGGu 5' hsa-miR-34a
      | || | | |||||
5' uuuuaCUGCCACUAAACUGCCu 3' E2F5 (+1445 to +1466)

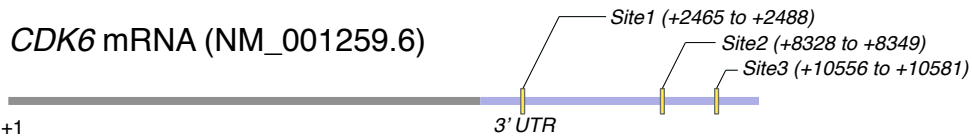
```

Site3: (M, T)

```

3' ugUUGGUCGAUUC-UGUGACGGu 5' hsa-miR-34a
      || ||| | | |||||
5' ccAAACAG--ACGUUCACUGCCa 3' E2F5 (+1489 to +1509)

```

D**Site1:** (M, T)

```

3' uguUGGUCGAU--UCUGUGACGGu 5' hsa-miR-34a-5p
      ||: ||: | |||||
5' uguACUUUCUGCCACACACUGCCu 3' CDK6 (+2465 to +2488)

```

Site2: (M, T)

```

3' uguuggucgauucugUGACGGu 5' hsa-miR-34a-5p
      |||||
5' uauaacuacauauugACUGCCa 3' CDK6 (+8328 to +8349)

```

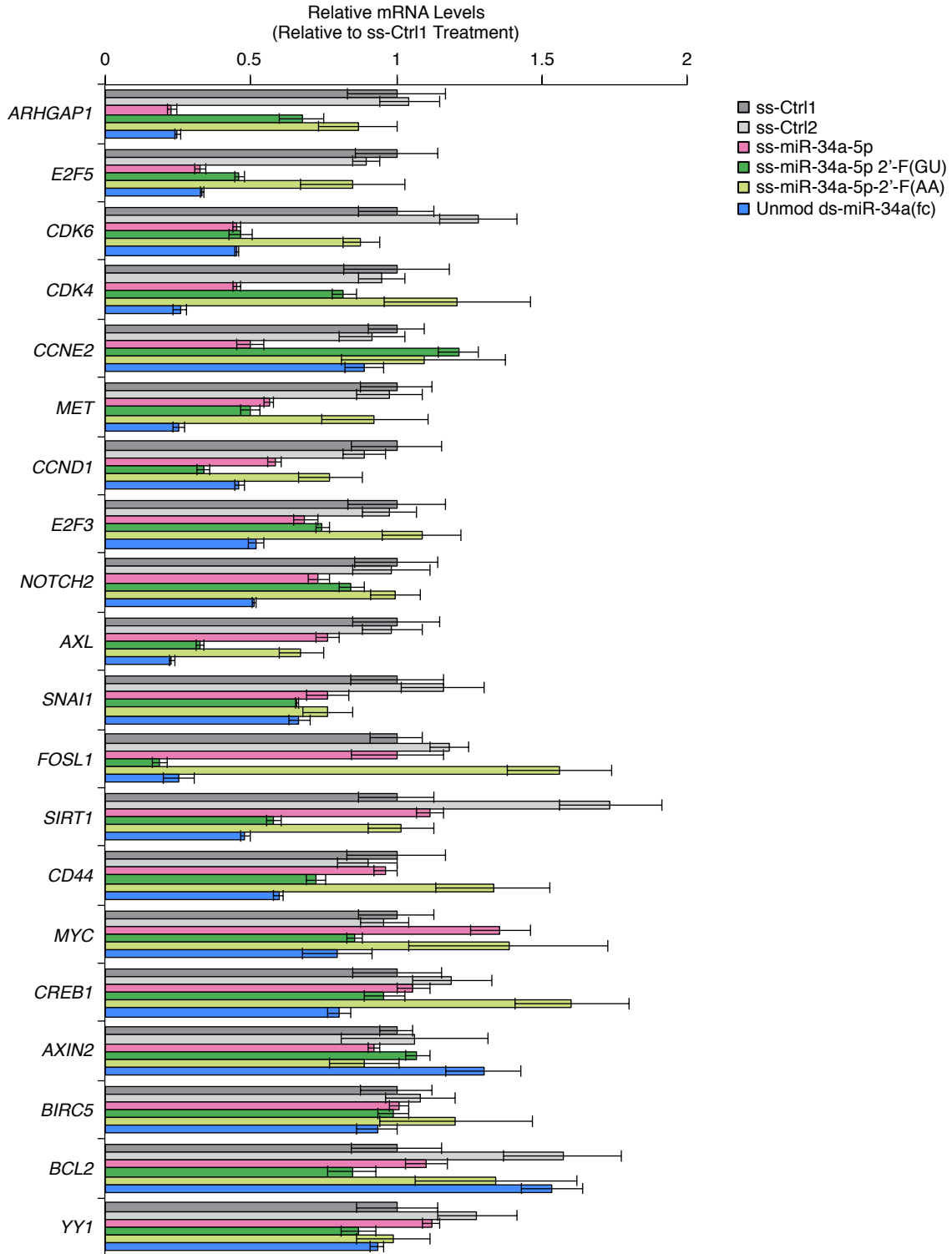
Site3: (M, T)

```

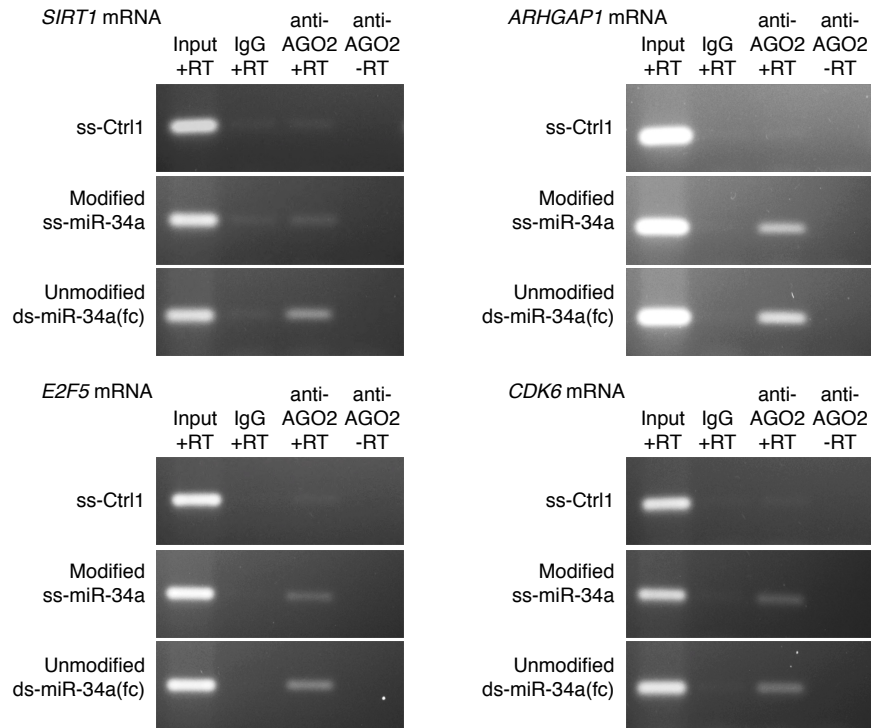
3' ugUUGGUCGAUUCU----GUGACGGu 5' hsa-miR-34a-5p
      || |||: :|| |||||
5' agAAGCAGUGUGAAAUUCACUGCCu 3' CDK6 (+10556 to +10581)

```

Supplementary Figure S1. Potential target sites for miR-34a-5p on the 3' UTRs of *SIRT1*, *ARHGAP1*, *E2F5*, and *CDK6* mRNAs. Two web-based programs “microRNA.org: microRNA Targets & Expression Profiles, August 2010 release” (<http://www.microrna.org/microrna/home.do>, cBio, the Computational Biology Center at Memorial Sloan-Kettering Cancer Center (MSKCC)) and “TargetScan release 7.0” (<http://www.targetscan.org>; Dr. David Bartel’s Lab/Whitehead Institute/MIT/HHMI) were used for predicting miR-34a-5p target sites. The predictions of base pairings shown in panel **A–D** are the results from microRNA.org. (M): Target sites predicted by microRNA.org; (T): Target sites predicted by TargetScan.



Supplementary Figure S2. qPCR data showing the effect of miR-34a mimics on mRNA expression of 20 different miR-34a target genes. Ss- and ds-miR-34a mimics and control oligomers (ss-Ctrl1, ss-Ctrl2) were transfected into HeLa cells at 50 nM using Lipofectamine RNAiMAX. Cells were harvested two days after transfection for qPCR analysis. Values are means \pm SD. n=4.



Supplementary Figure S3. Analysis of qPCR products from AGO2-RIP experiments by agarose gel electrophoresis. Before qPCR, each cDNA sample from AGO2-RIP experiments was appropriately diluted for normalization based on qPCR data for each input sample. After 31 (*SIRT1* mRNA), 30 (*ARHGAP1* mRNA, *E2F5* mRNA), or 29 (*CDK6* mRNA) cycles of qPCR, qPCR products were purified by ethanol precipitation and then analyzed by 1.5% agarose gel electrophoresis. The gels were stained with ethidium bromide. +RT: with reverse transcription, -RT: without reverse transcription.

Supplementary Table S1. Sequences of duplex RNAs used in this study.

Strand Name	dsRNA Name	Sequence (5' → 3')	Target
ds-Ctrl (sense)	ds-Ctrl	UCGAAGUAAUCCGCGUACGdTdT	(Non-complementary Negative Control)
ds-Ctrl (antisense)		CGUACGCGGAAUACUUCGAdTdT	
siAGO1 (sense)	siAGO1	GGAGUUACUUUCAUAGCAUUU	<i>AGO1</i> mRNA
siAGO1 (antisense)		pAUGCUAUGAAAGUAAUCUUCU	
siAGO2-1 (sense)	siAGO2 (pool)	GCACGGAAGUCCAUCUGAAUU	<i>AGO2</i> mRNA
siAGO2-1 (antisense)		pUUCAGAUGGACUCCGUGCUU	
siAGO2-2 (sense)		GCAGGACAAAGAUGUAUUUUU	
siAGO2-2 (antisense)		pUAAUACAUCUUUGUCCUGCUU	
siAGO2-3 (sense)		GGGUCUGUGGUGAUAAAUAUU	
siAGO2-3 (antisense)		pUAUUUAUCACCACAGACCCUU	
siAGO2-4 (sense)		GUAUGAGAACCCAAUGUCAUU	
siAGO2-4 (antisense)		pUGACAUUGGGUUCUCAUACUU	
siSirt1 (sense)	siSirt1	AGCUGGGGUGUCUGUUUCAdTdT	<i>SIRT1</i> mRNA
siSirt1 (antisense)		UGAAACAGACACCCAGCUdTdT	

siRNA sequences are listed 5' to 3'. The oligomers have two dT or U overhangs at their 3' end. siAGO2 was used as a pool of 4 different siAGO2 at total 25 nM (siAGO2-1 (6.25 nM) + siAGO2-2 (6.25 nM) + siAGO2-3 (6.25 nM) + siAGO2-4 (6.25 nM)). p: phosphate.

Supplementary Table S2. Sequences of qPCR primers used in this study.

Strand Name	Sequence (5' → 3')	Strand Name	Sequence (5' → 3')
ARHGAP1 ex3F	GGAACCTGTCCACACACCTGAA	YY1 ex2F	GAATACCTGGCATTGACCTCTC
ARHGAP1 ex4R	ACAATGATCTTCCGCCCATAC	YY1 ex3R	TGTTCTTGGAGCATCATCTTCTT
E2F5 ex2F	CTGCTGATACTTTGGCTGTGAG	P4HA1 ex5F	ACGCTCCAGGATACCTACAA
E2F5 ex3R	ATTACAGCCAGCACCTACAC	P4HA1 ex6R	CTGCTTCTGTATAGGCCACTTT
CDK6 ex3F	CTTGGATAAAGTTCCAGAGCCT	CCAR1 ex11F	GCACAGAGAAGTAGAGTCCTTAGA
CDK6 ex4R	TTTAGATCGCGATGCACTACTC	CCAR1 ex12R	TCAGCAAGAGCACATGACTTAT
CDK4 ex3F	GTAGACCAGGACCTAAGGACATA	CHKA ex1F	CTATCTGTGGTGCAAGGAGTT
CDK4 ex4R	TGCAATTGGCATGAAGGAAATC	CHKA ex2R	GGAACAGCATGTTGCTAAGG
CCNE2 ex5F	TTATTAATCCTTACCTTTGCC	MTOR ex7F	CGGTGTTGCAGAGACTTGAT
CCNE2 ex6R	GAAGTATGGACCTCATCTGTG	MTOR ex8R	TCATTTGGATCAGCGAGTTCTT
MET ex6F	GGAAGTTGGATTTCGGAGGAATA	TPD52L2 ex2/3JF	CTTACCAAGGTGGAAGAGGAAAT
MET ex7R	AGGACCAACTGTGCATTTCA	TPD52L2 ex3R	CTCCTGGACAGGTTCTGTTTC
CCND1 ex2F	GTTTCGTGGCCTCTAAGATGAA	HMGA2 ex3F	CAAGAGGCAGACCTAGGAAATG
CCND1 ex3R	AGGTTCCACTTGAGCTTGTT	HMGA2 ex5R	GCAGACTCTTGTGAGGATGTC
E2F3 ex2F	GCACTACGAAGTCCAGATAGTC	FIGN ex2F	GGAATTCCTGCTTGTGAGAAATC
E2F3 ex3R	GGCTCAGGAGCTGAATGAA	FIGN ex3R	GTGATGTCAAAGTGCTGTTCTG
NOTCH2 ex7F	GCTGACTGCACAGAAGATGT	PLAGL2 ex2F	AGACCATATAGCTGCCCTCA
NOTCH2 ex8R	CTTCAGACACTCACAGTGAAG	PLAGL2 ex3R	TCCTTGCGGTGAAACATCTTA
AXL ex10F	GTCCTCATCTTGGCTCTCTTC	NRAS ex4F	CCAAGAGTTACGGGATTCCATT
AXL ex11R	GACTACCAGTTCACCTCTTTCC	NRAS ex5R	CGGTAAGTGGCGTATTTCTCTTAC
SNAI1 ex1/2JF	GCAGGACTCTAATCCAGAGTTTAC	SOX9 ex1/2JF	GCAAGCTCTGGAGACTTCTG
SNAI1 ex2R	AGGACAGAGTCCCAGATGAG	SOX9 ex2R	CGCGGCTGGTACTTGTAAATC
FOSL1 ex3F	GGAAGGAACTGACCGACTTC	EIF2C1(AGO1)	Hs00201864_m1 (ABI)
FOSL1 ex4R	CTTCTGCAGCTCCTCAATCT	EIF2C2(AGO2)	Hs00293044_m1 (ABI)
SIRT1 ex5F	TGCGGGAATCCAAAGGATAAT	h18S F	GACCAGAGCGAAAGCATTG
SIRT1 ex6R	TCGTACAGCTTCACAGTCAAC	h18S R	TCGGAACTACGACGGTATCT
CD44 ex2F	ACCTCTGCAAGGCTTTCAATA		
CD44 ex3R	CACCACGTGCCCTTCTATG		
MYC ex2F	TCGGATTCTCTGCTCTCCT		
MYC ex3R	CAACATCGATTTCTTCTCATCTTC		
CREB1 ex4F	TCACAGGAGTCAGTGGATAGT		
CREB1 ex5R	CCTGGTGCATCAGAAGATAAGT		
AXIN2 ex2F	CGGAAACTGTTGACAGTGGATA		
AXIN2 ex3R	TGGCTGGTGCAAAGACATAG		
BIRC5 ex3F	GCGCTTTCCTTTCTGTCAAG		
BIRC5 ex4R	CTCCGCAGTTTCTCAAATTC		
BCL2 ex2F	GCCCTGTGGATGACTGAGTA		
BCL2 ex3R	GCCAGGAGAAATCAAACAGAGG		