

Supplementary Materials

Supplementary Table 1. Conserved miRNAs predicted to target both 5' and 3' UTR 8-mers.

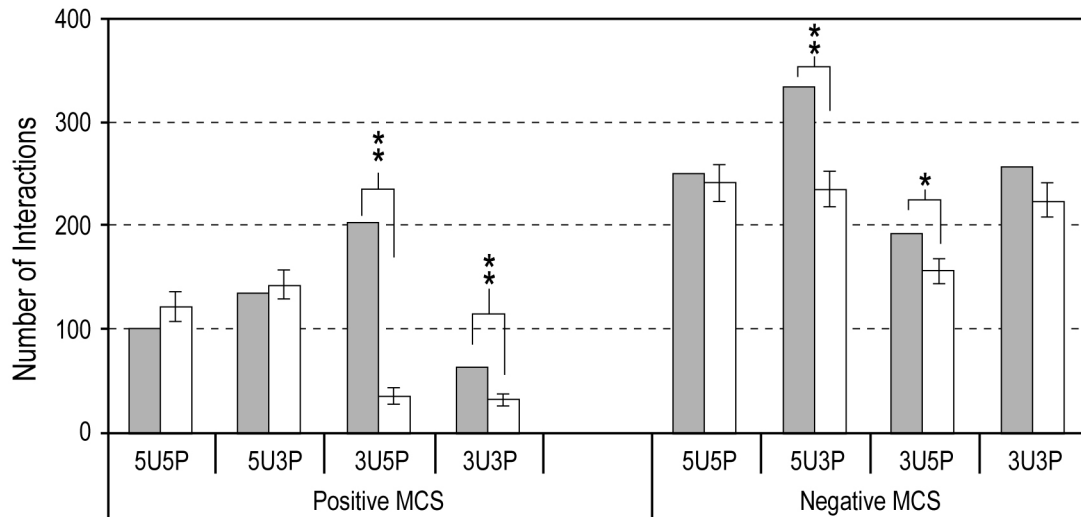
miRNA	5' UTR Motif ¹	3' UTR Motif ¹
<i>hsa-let-7i</i>	GGCGGCAC (0, 48)	CTACCTCA (139, 263), ACTACCTC (63, 160), TTACCTCA (99, 262), GCTACCTC (48, 152), ATTACCTC (40, 156)
<i>hsa-miR-16</i>	GCCAGTGT (1, 53)	TGCTGCTA (124, 380)
<i>hsa-miR-17</i>	GCCTGCAC (0, 66), CCTGCACT (1, 63)	AGCACTTT (190, 601), GCACTTTG (119, 398), AAGCACTT (123, 483), GGCACTTT (64, 276)
<i>hsa-miR-18b</i>	GACTGCGC (0, 54)	GTGCCTTA (147, 271), GCACTTTA (193, 405), TGCACTTT (244, 596), GCACTTTG (119, 398), ATGCACTT (82, 302)
<i>hsa-miR-20a</i>	GCCTGCAC (0, 66), CCTGCACT (1, 63)	GCACTTTA (193, 405), AGCACTTT (190, 601), AAGCACTT (123, 483), GGCACTTT (64, 276)
<i>hsa-miR-20b/93</i>	GCCTGCAC (0, 66), CCTGCACT (1, 63)	AGCACTTT (190, 601), GCACTTTG (119, 398), GGCACTTT (64, 276)
<i>hsa-miR-25</i>	TCAGGCCG (0, 37), CGGGCTGA (1, 69), GACTGAGA (1, 57),	AGTGCAAT (90, 273), GGTGCAAT (33, 116), AAGTGCAA (81, 306),

	GACCGGGG (1, 50)	GTGCAATG (46, 183)
<i>hsa-miR-93</i>	GCCTGCAC (0, 66),	AGCACTTT (190, 601), GCACTTTG (119, 398), GGCACTTT (64, 276)
<i>hsa-miR-106a</i>	GCCTGCAC (0, 66), CCTGCACT (1, 63)	AGCACTTT (190, 601), AAGCACTT (123, 483), GGCACTTT (64, 276)
<i>hsa-miR-125a-3p</i>	CTCCTAGG (0, 47)	GCCTTACT (36, 172)
<i>hsa-miR-128</i>	AGGGGACC (1, 64), GAGACCGG (1, 49)	CACTGTGA (132, 414), TACTGTGA (99, 354)
<i>hsa-miR-133b</i>	TGGCTGGT (1, 51)	GGGACCAA (44, 214)
<i>hsa-miR-139-5p</i>	TGGGGGCA (1, 63)	GTACTGTA (136, 338), TGTACTGT (129, 506), TGCACTGT (90, 418), GTACTGTG (55, 265)
<i>hsa-miR-196a/b</i>	CCCAGCGA (1, 57)	AACTACCT (43, 194)
<i>hsa-miR-199a-3p</i>	GCCAGTGT (1, 53)	ACTACTGT (59, 268)
<i>hsa-miR-199b-3p</i>	GCCAGTGT (1, 53)	ACTACTGT (59, 268)
<i>hsa-miR-202</i>	TCTGTGCC (1, 74)	TATACCTC (35, 161)
<i>hsa-miR-330-5p</i>	CTGAGACA (1, 60)	CTCAGGGA (129, 451)
<i>hsa-miR-362-5p</i>	CTTGCACC (0, 37)	TTCAGGGA (81, 395)
<i>hsa-miR-373</i>	GCCCTGAA (0, 56), CCCCAAGA (1, 49)	AGCACTTT (190, 601), AAGCACTT (123, 483), GGCACTTT (64, 276)
<i>hsa-miR-503</i>	TGCGGGAC (0, 47),	TGCTGCTA (124, 380),

	AGAGCTGT (1, 54)	TTGCTGCT (145, 664)
<i>hsa-miR-506</i>	TGCTCGGG (0,45)	GTGCCTTA (147, 271), GTGCCTTG (133, 355), GGTGCCTT (82, 281), GGTGCTTT (85, 340)
<i>hsa-miR-509-3p</i>	CCCGCAGA (0, 55), ACTCACAG (0, 46), TCTACAGA (0, 38), CCCACAGG (1, 66)	GTGCCAAT (40, 142)
<i>hsa-miR-517a/b</i>	GCGCTCTG (1, 61)	GTGCATGA (38, 170)
<i>hsa-miR-517c</i>	GCGCTCTG (1, 61)	GTGCATGA (38, 170)
<i>hsa-miR-519a</i>	GCGCTCTG (1, 61)	TGCACTTT (244, 596), ATGCACTT (82, 302)
<i>hsa-miR-520a-3p</i>	AGTCCAGA (1, 52)	AAGCACTT (123, 483)
<i>hsa-miR-520d-3p</i>	ACCCACCA (0, 38), CTCGCCGA (0, 37), CGCCAGAG (1, 59)	AAGCACTT (123, 483)
<i>hsa-miR-520g</i>	GCGCTCTG (1, 61)	AGCACTTT (190, 601), GCACTTTG (119, 398), AAGCACTT (123, 483), GGCACTTT (64, 276)
<i>hsa-miR-524-3p</i>	CCGGAGGG (1, 90)	GTGCCTTT (179, 482), GTGCCTTC (107, 361), AGTGCCTT (227, 473), GGTGCCTT (82, 281) AAGTGCCT (196, 430),
<i>hsa-miR-608</i>	GCTGTCCT (0, 70), CGGGGTTG (0, 41)	ACTACCTC (63, 160), CTACCTCT (96, 284),

		GCTACCTC (48, 152)
<i>hsa-miR-613</i>	GGCGAAGG (0, 64), GGCAAGGG (1, 56)	ACATTCCT (85, 405)
<i>hsa-miR-650</i>	CTGAGGGT (0, 67), TCTGAGGG (1, 75)	GTTGCCTT (66, 319)
<i>hsa-miR-652</i>	GCGACCCT (0, 46), CGACCCTG (0, 44)	TGGTGCTA (116, 272), GGTGCTAT (59, 167), GTGCCATT (61, 277)
<i>hsa-miR-661</i>	TGCGGGCC (0, 59), CGCGTGGG (0, 43), CGTGGGCC (1, 61)	CTCAGGTA (39, 156), ACTCAGGT (37, 175)
<i>hsa-miR-770-5p</i>	GGTTCTGA (0, 44), CTTGCGGC (1, 52), CCTTGGCA (1, 52)	TGGTGCTG (118, 525)
<i>hsa-miR-885-3p</i>	GTCCGCTG (0, 38), TCCACTGC (1, 59), CCACTGCA (1, 58)	TTGCTGCT (145, 664)

¹Numbers within parentheses indicate conserved instances and number of human occurrences.



Supplementary Figure 1. Analysis of predicted interactions based on conservation score. Closed bars indicate the number of predicted interactions between 5'-UTR or 3'-UTR 8-mers (5U and 3U respectively) and 5'- or 3'-ends of mature human miRNAs (5p and 3p respectively). Open bars indicate mean number of interactions using shuffled 8-mers after 1000 shuffling iterations. Double asterisk indicates $p < 5e-05$ and single asterisk, $p < 5e-03$.

Sequences used in human *AXIN2* assay

AXIN2 5'-UTR (NM_004655). Sequence cloned upstream of the luciferase coding

region is shown in italics with predicted overlapping binding sites for the 3'-end of *miR*-

34a shown in bold blue italics.

cggctgtgattggcgcggcggggatcactggctccgcgagcctg***gcccgggggagtcggc tggagc
cggctgcgctttgataaggtcctggcaactcagtaacagcccgagagccgggaaataaaaaataac
ccctcagagcgatggatttcggggccgcccggcgccgagggcggccgccaaggccctgctgtaa
aagagaggagggttcagatgagcccctgctgacttgagagagacagagagaccacgccgattgctg
agaggaactggaagaagaaaaattcccagactcagtgggaagagctccctcacc***

AXIN2 3'-UTR (NM_004655). Seed match sites are shown in bold underlined red and

3'-end interaction sites in blue italics. Seed match site with no GU wobble is in caps.

gccctgggggtctggctttggtgaactggtggagcccgaagctcttgtgaactgtcttggctgtga
gcaactgcgacaaaacattttgaaggaaaattaaaccaatgaagaagacaaagtctaaggaagaa
t***cggccagt***gggcttcgggagggcggggggaggttgattttcatgattcatgagctgggtactg
actgagataagaaaagcctgaactatttattaaaaacatgaccactcttggctattga***agatgct
gcc***tgattttgagag***ACTGCCA***tacataatatatgacttccttaggatctgaaatccataaaacta
agagaaaactgtgtatagcttacctgaacaggaatccttactgatatttatagaacagttgatttc
ccccatccccagtttatggat***atgctgct***ttaaacttggaagggggagacaggaagttttaattg
ttctgactaaacttaggagttgagctaggagtgcgttcatggtttcttactaacagaggaatta
tgctttgactacgtccctccaagtgaagacagactgttttagacagactttttaaaatggtgcc
ctaccattgacacatgcagaaattggtgctgttttttttttttcttatgctgctctgttttg
tcttaaagggtcttgagggttgaccatggtgctcatcatcaacattttgggggttggttggtg
ggatgatctgttgagagggagaggcaggggaaccctgctccttcgggcccaggttgatcctgtg
actgaggctccccctcatgtagcctccccaggcccaggccctgaggcctgctagaat***cactgcc
g***ctgtgctttcgtggaaatgacagttccttggtttttttgtttctgtttttgttttacattagtc
attggacc***acagcca***ttcaggaactacccccctgccccacaaagaaatgaacagttgtagggagac
ccagcagcacctttcctccacacaccttcattttgatgttcgggtttttgtgttaagttaattctg
tacattctgtttgccattgttacttgactatacatctgtatatagtgtacggcaaaagagtatt
aatccactatctctagtgttgacttttaaatcagtagctacgtgtacctgcacgggtcaccgct
ccgtgtgtcgccttatattgagggctcaagctttcccttggttttttgaaaggggtttatgtataa
atatattttatgcctttttattacaagtcttgactcaatgacttttgtcatgacattttgttct
acttatactgtaaattatgcattataaagaggttcattttaaggaaaattacttggtacaataatta
ttgtaattaagagatgtagcctttattaaaattttatatatttttcaaaa

Multiple alignment of Axin2 5'-UTR sequences from 4 mammalian species (CLUSTAL 2.0.8 multiple sequence alignment). Predicted 5'-UTR target sites for the 3'-end of miR-34a are shown in bold blue italics.

Human	-----CGGCTGTGATTGGCGCGGCGGGATCACTGGCTCCGCGAGC	40
Dog	-----	
Mouse	GCGCGGCGGGATCACTGGCTCCCCGAGCCCGGCCCGGGGG AGTCGGCTGGAGCCGGCTGC	60
Rat	-----	
Human	CTGGCCCGGGGG AGTCGGCTGGAGCCGGCTGC CGCTTTGATAAGGTCCTGGCAACTCAGTA	
100		
Dog	-----	
Mouse	GCTTTGATAAGGTCCTGGCAACTCAGTAACAGCCCAAGAACCGGGAAATAAAAATAAGCA	
120		
Rat	-----	
Human	ACAGCCCGAGAGCCGGGAAATAAAAATAACCCCTCAGAGCGATGGATTTCTGGGGCCGCCC	
160		
Dog	-----	
Mouse	GCCGTTTCGCGATGGATTTCTGGGGCCACCCGGAGGCCGAGGCGTCCGCCTCCCCAAAGGAG	
180		
Rat	-----	
Human	GGCGGCCGAGGCGCCCGCCGAAGGCCCTGCTGTAAAAGAGAGGAGGTTTCAGATGAGCCCC	
220		
Dog	-----	
Mouse	AGCTTTGCTGTAAAAGAGAGGAGGCTCACATGAGCCCCTGCTGACTTAAGAGAGACCAAG	
240		
Rat	-----	
Human	TGCTGACTTGAGAGAGACAGAGAGACCACGCCGATTGCTGAGAGGAACTGGAAGAAGAAA	
280		
Dog	-----GAGAGAGAAAGAGAGACCACGCTGATTGCTGAGAGGAACTGGGGGAAGGGA	51
Mouse	CCGATTGCTGAGAGGAACTGGAAGAAGAAAAAGGAGGAGGAGGAAAA-AAAGCAAAACA	
299		
Rat	-----TGAGAGGAACTGGAAGAAGAAAAAGGAAGAGGAAAAAAAA-AAAGCAAAACA	51
	***** * * *** * * * ** ** ** *	
Human	AATTCCCAGACTCAGTGGGAAGAGCTCCCTCACC	314
Dog	ACAAACAACTCCGTCGCGAAGAACTCCCTCACC	85
Mouse	AAATCCAACTCAGT-GAGACGCTCTCCCTCACC	332
Rat	AAACCCAACTCAGT-GAGACGCTCTCCCTCACC	84
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Multiple alignment of Axin2 3'-UTR sequences from 4 mammalian species (CLUSTAL

2.0.8 multiple sequence alignment). Predicted seed match sites are shown in bold red and

the 3'-end interaction sites in blue italics.

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Human  -----GCCCTGGGGTCTGGCTTTGGTGAAGTGTGGAGCCCGAAG 40
Dog    -----GCCTCGGGGGTCTGGGCCCCGGCGGACGC---GGGGCCACCG 38
Mouse  CCTTGGCCTCCTCGGCGTGCAACCTGGGCAAGCACCTCGGCGTGCACCATGGAGCCGAAG 60
Rat    CCTCGGCCTCCGCCGCGTGCA-CCTCCGCGTGCACCTCCGCGTGCACCACGGAGCCGGAG 59
          *      *          *      * * *      *      **      *

Human  CTC-TTGTGAAGTGTCTTGGCTGTGAGCAACTGCGACAAAACATTTTGAAGGAAAATTAA 99
Dog    CCC-GCGCCACCAGCCTGGGCCATGACCGACCGACGAGACCTTTTGAAGGAAAACGAA 97
Mouse  CCCAGAGAC-CCTGTCTCAGGCCTACGCAACAGCCACGAAATATTCTGAAGGAAAATGAA 119
Rat    CCCAGAGAGACCTGTCTCAGGCCTACACAACAGCCATGAAATATTTTGAAGGAAAATGAA 119
          * *      *      * * * *      *      * * * * * * * * * * * * * * * * *

Human  ACCAATGAAGAAGACAAAGTCTAAGGAAGAATCGGCCAGTGGGCCTTCGGGA-----GG 153
Dog    ACCAATGAAGAAGACAGAGTCTAGGGAAGACTTGGCCACTGGCCACGCGGGGAGGGCGGG 157
Mouse  ACCAATTAAGAAGACAAAGCCTAGGGAGGGACTGGCGCCTGGGCCTTCAGGA-----G 172
Rat    ACCAATTAAGAAGACAAAGCCTAGGGAGGGACTGGCCCCTGGGCCTTCAGGA-----G 172
          * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Human  GCGGGGGGAGGTTGATTTTCATGATTCATGAGC-TGGGTACTGACTGAGATAAGAAAAGC 212
Dog    GAGGGGGGAGGTTGGTTTTTCATTATTCACGAGC-TGGGTACT----GAGATAAGAAAAGC 212
Mouse  GGCGGGGGTAGTTGATCTTCAGTCTCCAGGAGCCTGGGTACC----GAGATGAGAAAAGC 228
Rat    GGCGGGGGTGTTGGTTTTCAATATCCACGAGC-TGGGTACT----GAGATCAGAAAAGC 227
          *      * * * * * * * * * * * * * * * * * * * * * * * * * * *

Human  CTGAAGTATTTATTTAAAAACATGACCACTCTTGGCTATTGAAGATGCTGCTTGTATTTGA 272
Dog    CTGAAGTATTTATTTAAAAACATGACCACTCTTGGCTATTGATGATGCTGAGTTGTATTTGA 272
Mouse  CTGAAGTATTTATTTCAAACATGACCACTCTGGGCTATAGAAGATGCTGAGTTGTGTTTGA 288
Rat    CCGAAGTATTTATTTCAAACATGACCACTCTGGGCTATAGAAGATGCTGAGTGC--TCGA 285
          *      * * * * * * * * * * * * * * * * * * * * * * * * * * *

Human  GAGACTGCCATACATAATATATGACTTCCTAGGGATCTGAAATCCATAAACTAAGAGAAA 332
Dog    GAGACTGCCATACATAATATATGACTGCCTAGGGATCTGAAATCCATAAACTAAGAGAAA 332
Mouse  GAGACTGACATACATAATAGATGACTTCCTAGGGTTCTGAAATTCATAGACTAAGAGAAA 348
Rat    GAGACTGCCATACATAATAGATGGCTTCCTAGGGATCTGAAATCCATAGACTAAGAGAAA 345
          * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Human  -CTGTGTATAGCTTACCTGAACAGGAATCCTTACTGATATTTATAGAACAGTTGATTTC 391
Dog    -CTGTGTATAGCTTACCTGAACAGGAGTCCTTACTGCTATTTATTGAACAATTGATTCCC 391
Mouse  ACTGTGTATAGCTTGCCCGAACAGGAGTCCTTACTGATATTTATTGAACAGTCGATTCCC 408
Rat    ACTGTGTATAGCTTGCCCGAACAGGAGTCCTTACTGATATTTATTGAACAGTCGATTCCC 405
          * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

Human  CC-----CA-TCCCCAGTTTATGGAT-ATGCTGCTTT-AAACTTGGAAG 432
Dog    CC-----CAGCCCCAGTTTATGGAT-ATGCTGCTTT-AAACACGGAAG 433
Mouse  CTACCCGCC-----CTCCCTACCCCCAGCCCCAGTTTATGCTGCTTTTAAACCTGGAAG 463

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Human	CC-CCTGCCCCACAAAGAAATGAACAGTTGTAGGGAGACCCAGCAGCACC-TTTCCTCCA	995
Dog	CCGCTTGGCCCTGCAAAGAAGCGGACAGTTGTGGGAAGACCTAGCAGCACCCCTTTCCTCCA	1007
Mouse	CC-----TCGCCCACCTGACCCTGCGAGGACACGGGCACCCAGCAGCACCTCTCCTCTGT	968
Rat	-----	
Human	CACACCTTCATTTTGATGTTTCGGGTTTTTGTGTTAAGTTAATCTGTACATTCTGTTTGCC	1055
Dog	GACACCTTCATTTTGACGTTTCGGGTTTTTGTGTTAAGTTAATCTGTACATTCTGTTTGCC	1067
Mouse	TACCTTCCCCTTGGCGATCGCTCGGGTTTTGTGTTAAGTTAATCTGTATGTCCTGTCTGCC	1028
Rat	-----	
Human	ATTGTTACTTGTACTATACATCTGTATATAGTGTACGGCAAAAGAGTATTAATCCACTAT	1115
Dog	ATTGTTACCTGTACTATACGTCTGTATATATTGTACGACAGAAGAGTATTAATCCACTAT	1127
Mouse	AGCGTTCCCTGTACTATAGGCCTGTGTATAGTGTAGGGCA---GAGCGTTGACCCACTGG	1085
Rat	-----	
Human	CTCTAGTGCTTGACTTTA-AATCAGTACAGTACCTGTACCTGCACGGTCACCCGCTCCGT	1174
Dog	CTCTAGTGCTTGACTTGA-AATCAGTACAGTACCTGTACCTGCACGGCGCCCCGCTCCGT	1186
Mouse	CT--AGTGCTTGACTTGGGAATCAGGACAGTACCTGTACAGGCACGGGGACCCGCTCCGT	1143
Rat	-----	
Human	----GTGTCGCCCTATATTGAGGGCTCAAGCTTTCCTTGTTTTTTGAAAGGGGTTTATG	1230
Dog	----GTGTCGCCCTATATTGAGGGCTCCAAGCTTTCCTTGTTTTTTGAAAGGGGTTTATG	1242
Mouse	CCGTGCGCGCCCTATATTGAGGGCTCCAGCTCTCCCTTGTTTTTTGAAAGGGGTTTATG	1203
Rat	-----	
Human	TATAAATATATTTTATGCCTTTTTTATTACAAGTCTTGT-ACTCAATGACTTTTGTCTATGA	1289
Dog	TATAAATATATTTTATGCCTTTTTTATTACAAGTCTTGT-ACTCAATGACTTTTGTCTATGG	1301
Mouse	TATAAATATATTTTATGCCTTTTTTATTACAAGTCTTGTTACTCAATGACTTTTGTCTATGG	1263
Rat	-----	
Human	CATTTTGTCTACTT-ATACTGTAAATTATGCATTATAAAGAGTTCATTTAAGGAAAATT	1348
Dog	CATTTTGTCTACTT-ATACTGTAAATTATGCATTATAAAGAGTTCATTTAAGGAAAATT	1360
Mouse	CAGTTTGTCTACTTTAGACTGTAAATTATGCATTATAAAGAGTTCATTTAAGGAAAATT	1323
Rat	-----	
Human	ACTTGGTACAATAATTATTGTAATTAAGAGATGTAGCCTTTATTAAAATTTTATATTTTT	1408
Dog	ACTTGGTACAATAATTATTGTAATTAAGAGATGTAGCCTTTATTAAAATTTTATATTTTT	1420
Mouse	ACTTGGTACAATAATTATTGTAATTAAGAGATGTAGCCTTTATTAAAATTTTATATTTTT	1383
Rat	-----	
Human	CAAAA	1413
Dog	C----	1421
Mouse	C----	1384
Rat	-----	