Supporting Information

Azuma-Mukai *et al.* 10.1073/pnas.0800334105

```
hAgo1  M--EAGPSGAAAGAYLPPLQQVFQAPRRPGIGTVGKPIKLLANYFEVDIP
hAgo2  MYSGAGPALAPPAPPPPIQGYAFKPPPRPDFGTSGRTIKLQANFFEMDIP
hAgo3  M--------EIGSAGPAGAQPLLMVPRRPGYGTMGKPIKLLANCFQVEIP
hAgo4  M----------EALGPGPPASLFQPPRRPGLGTVGKPIRLLANHFQVQIP

hAgo1  KIDVYHYEVDIKPDKCPRRVNREVVEYMVQHFKPQIFGDRKPVYDGKKNI
hAgo2  KIDIYHYELDIKPEKCPRRVNREIVEHMVQHFKTQIFGDRKPVFDGRKNL
hAgo3  KIDVYLYEVDIKPDKCPRRVNREVVDSMVQHFKVTIFGDRRPVFDGKRSL
hAgo4  KIDVYHYDVDIKPEKRPRRVNREVVDTMVRHFKMTIFGDRQPGYDGKRNM

hAgo1  YTVTALPIGNERVDFEVTIPGEG-KDRIFKVSIKWLAIVSWRMLHEALVS
hAgo2  YTAMPLPIGRDKVELEVTLPGEG-KDRIFKVSIKWVSCVSLQALHDALS
hAgo3  YTANPLPVATTGVDLDVTLPGEGGKDRPFKVSIKFVSRVSWHLLHEVLTG
hAgo4  YTAHPLPIGRDRVDMEVTLPGEG-KDQTFKVSVQWVSVVSLQLLLEALAG

hAgo1  GQIPVPLE--------SVQALDVAMRHLASMR      171 a.a.
hAgo2                                        148 a.a.
hAgo3  RTLPEPLELDKPISTNPVHAVDVVLRHLPSMKY     174 a.a.
hAgo4  HLNEVPDD--------SVQALDVITRHLPSMRY     164 a.a.
```

Fig. S1. Amino acid sequence alignment of the N-terminal regions of hAgo1, hAgo2, hAgo3, and hAgo4. The regions were used as antigens for immunizing mice. The pink boxes indicate amino acids perfectly identical among all Argonautes. Amino acids highly conserved are highlighted with light green boxes.

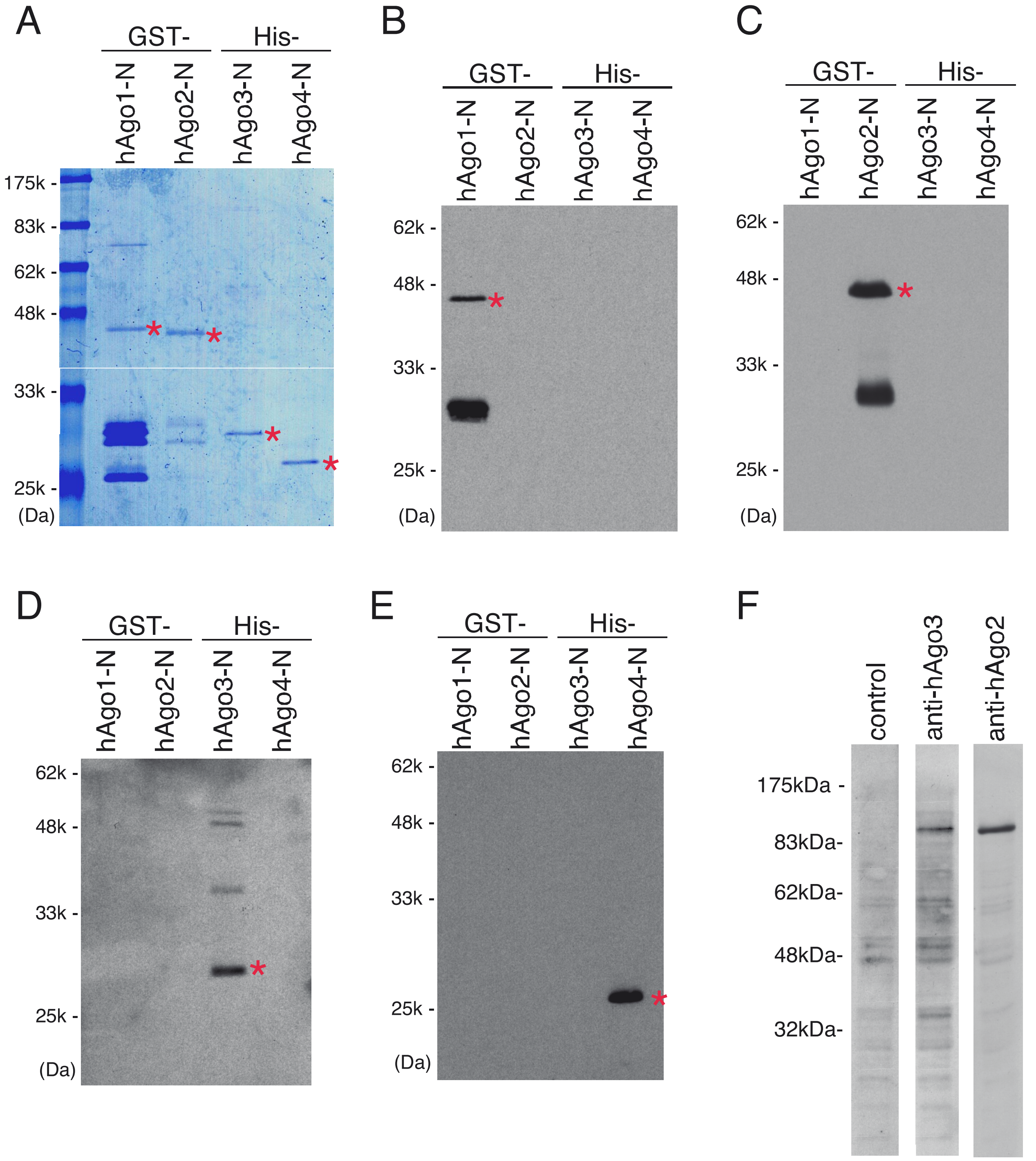


Fig. S2. Monoclonal antibodies against individual hAgos. (*A*) N-terminal regions of hAgo1 and hAgo2 fused to GST produced in and purified from *E. coli* (GST-hAgo1-N and GST-hAgo2-N). The N-terminal regions of hAgo3 and hAgo4 were fused to 6× His-tag and produced in and purified from *E. coli* (His-hAgo3-N and His-hAgo4-N). Red asterisks show the expected full-length fusion proteins of each hAgo. (*B*) One-tenth of purified proteins shown in *A* separated on SDS/PAGE gel and, after transferring to a membrane, probed with anti-hAgo1 monoclonal antibody. Only GST-hAgo1-N (red asterisk) is detected, indicating that anti-hAgo1 we raised is specific to hAgo1. (*C*) Blot prepared as in *B* and probed with anti-hAgo2 antibody. (*D*) Blot prepared as in *B* probed with anti-hAgo3 antibody. (*E*) Blot prepared as in *B* probed with anti-hAgo4 antibody. (*F*) Total lysates of Jurkat cells probed with anti-hAgo2 and -hAgo3 antibodies. The blot indicated as ''control'' was probed only with secondary antibody.

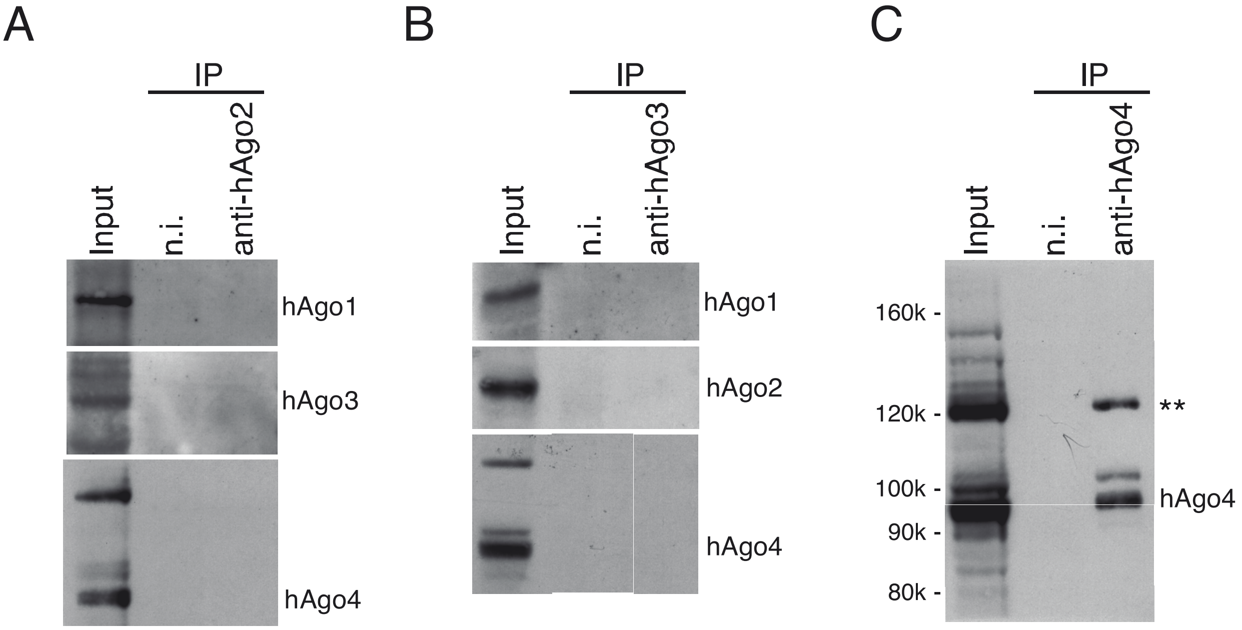


Fig. S3. Characterization of immunoprecipitates with anti-hAgo2, -hAgo3, and -hAgo4 antibodies from Jurkat cells. (*A*) Anti-hAgo2 immunoprecipitates (Fig. 1*A*) analyzed by Western blot analysis with anti-hAgo1, anti-hAgo3, and anti-hAgo4. (*B*) Anti-hAgo3 immunoprecipitates (Fig. 1*A*) analyzed by Western blot analysis with anti-hAgo1, anti-hAgo2, and anti-hAgo4. (*C*) Anti-hAgo4 immunoprecipitates (Fig. 1*A*) analyzed by Western blot analysis with anti-hAgo4. The 120-kDa band appearing in Fig. 1*A* (double asterisks) reacted with anti-hAgo4 even in Western blot analysis.

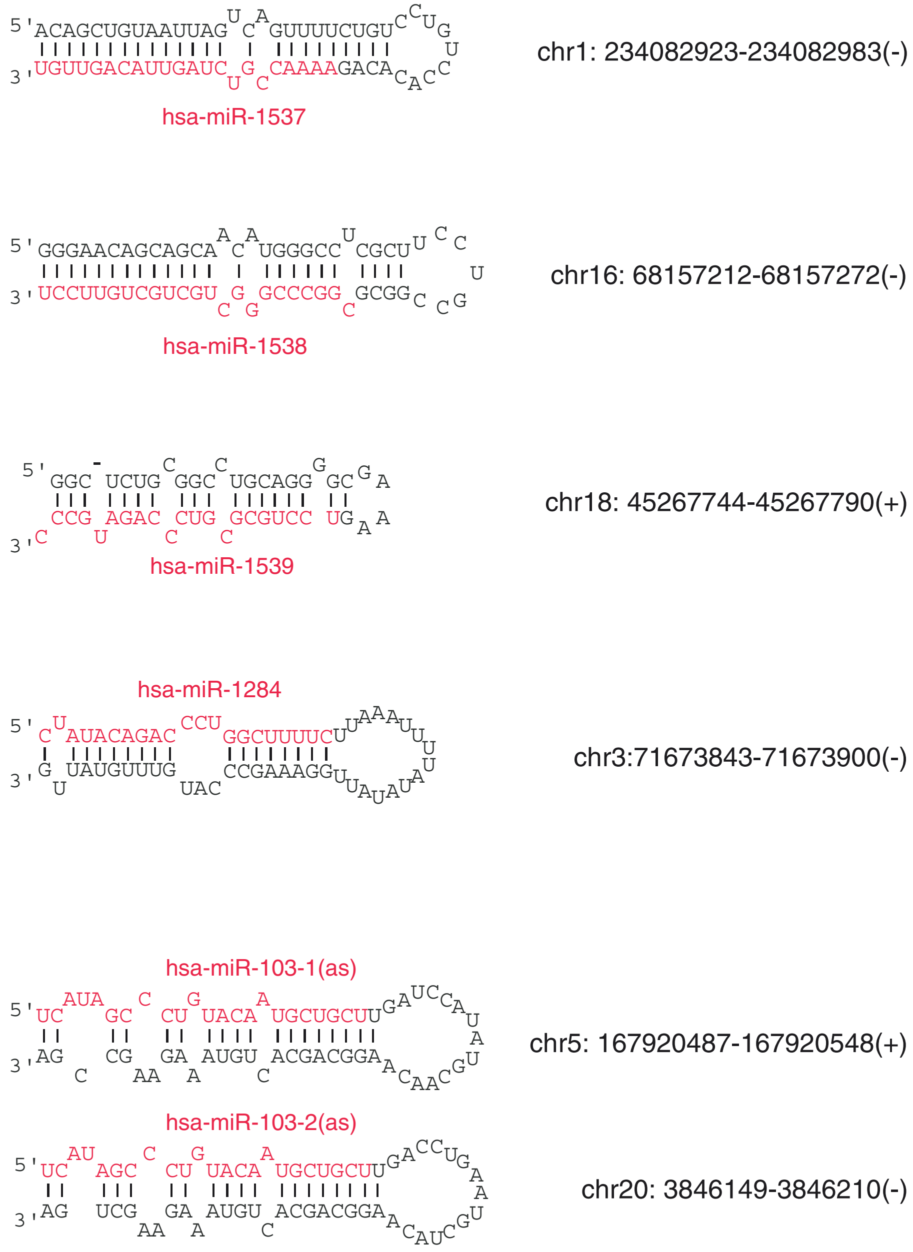


Fig. S4. Our sequence data analysis found five novel microRNA candidates (hsa-miR-20071, hsa-miR-20072, hsa-miR-20073, hsa-miR-2004, and hsa-miR-2005; temporal names). miR-20071 resides inside of lyst (lysosomal trafficking regulator isoform 1) intron, which is supported by a microRNA prediction (HS92) by Berezikov *et al.* (1). miR-20072 is inside of nfat5 (nuclear factor of activated T cells 5 isoform a) 5′ UTR, which is supported by a computational microRNA prediction (miRRim2837) by Terai *et al.* (2). miR-20073 is inside of rpl17 (putative NFκB activating protein) intron, which is supported by a prediction HS276 (1). miR-20074 is located inside of foxp1 (forkhead box P1 isoform 1) and is supported by a computational prediction miRRim999 (2). miR-20075 has a distinct feature: it is mapped onto known microRNA (miR-103-1) but its strand is opposite. miR-20075 precursors could be mapped onto chromosome 5 and chromosome 20, as indicated.

1. Berezikov E, *et al.* (2006) Diversity of microRNAs in human and chimpanzee brain. *Nat Genet* 38:1275–1277.
2. Terai G, Komori T, Asai K, Kin T (2007) miRRim: A novel system to find conserved miRNAs with high sensitivity and specificity. *RNA* 13:2081–2090.

```
hAgo2-top1        miR-150          5P


-------------UGUCUCCCAACCCUUGUACCAGU------------------------------------------------   11
--------------GUCUCCCAACCCUUGUACCAGUG-----------------------------------------------   13
--------------aUCUCCCAACCCUUGUACCAGUG-----------------------------------------------   64
---------------UCUCCCAACCCUcGUACCAGU------------------------------------------------   10
---------------aCUCCCAACCCUUGUACCAGUG-----------------------------------------------   11
---------------UCUCCCAACCCUcGUACCAGUG-----------------------------------------------   12
---------------gCUCCCAACCCUUGUACCAGUG-----------------------------------------------   13
---------------UCUCCCAACCCUUGcACCAGUG-----------------------------------------------   18
---------------UCUCCCAACCCUUGUACCAGUa-----------------------------------------------   23
---------------UCUCCCAACCCUUGUACCAGUu-----------------------------------------------   31
---------------UCUCCCAAuCCUUGUACCAGUG-----------------------------------------------   35
---------------UCUCCCAACCCUUuUACCAGUG-----------------------------------------------   47
---------------UCUCCCAACCCUUGUACCAGUGC----------------------------------------------   141
---------------UCUCCCAACCCUUGUACCAGUGa----------------------------------------------   362
---------------UCUCCCAACCCUUGUACCAGU------------------------------------------------   381
---------------UCUCCCAACCCUUGUACCAGUGu----------------------------------------------   530
---------------UCUCCCAACCCUUGUACCAGUG-----------------------------------------------   4478
----------------CUCCCAACCCUUGUACCAGUGu----------------------------------------------   14
----------------CUCCCAACCCUUGUACCAGUG-----------------------------------------------   167
-----------------UCCCAACCCUUGUACCAGUG-----------------------------------------------   11

CUCCCCAUGGCCCUGUCUCCCAACCCUUGUACCAGUGCUGGGCUCAGACCCUGGUACAGGCCUGGGGGACAGGGACCUGGGGAC
.((((((.((((((((((((((..(((.(((((((.((((....))).).))))))))))..)))))))))))).)))))))).
```

Fig. S5. miRNA sequence variations found in the hAgo2 and hAgo3 small RNA libraries. Variants of the top 30 miRNAs in both libraries are shown. For individual miRNAs, only variants that appeared a total of 10 times or more in the hAgo2 and hAgo3 small RNA libraries are listed. Nucleotide sequences shown on the bottom in each case are precursor sequences of the miRNAs, in which authentic mature miRNA sequences registered in databases are highlighted in yellow. Sequences in red (upper) are authentic mature miRNA sequences registered in databases. Additional nucleotides found in the variants of the 5′ and 3′ ends compared with the authentic miRNAs are shown in colors. Letters with green boxes indicate nucleotides most likely added to mature miRNAs after miRNA maturation processing (nontemplated extensions), whereas letters with purple boxes indicate nucleotides most likely originating from precursors together with authentic mature miRNAs (alternative ends), because those nucleotides completely match to the precursor sequences. Numbers shown on the right show total appearances of each variant in the libraries.

hAgo2-top2   miR-99b   5P

```
-----aCACCCGUAGAACCGACCUUGCG------------------------------------------         85
------CACCCGUAGAACCGACCUUGuG------------------------------------------         12
------CACCCGUAGAACCGACCUUGCu------------------------------------------         12
------CACCCGUAGAAuCGACCUUGCG------------------------------------------         12
------CACCCGUAGAACCGAuCUUGCG------------------------------------------         13
------CACCCGUAuAACCGACCUUGCG------------------------------------------         15
------CACCCuUAGAACCGACCUUGCG------------------------------------------         17
------CACCCGUAGAACCGACuUUGCG------------------------------------------         17
------CACCCGcAGAACCGACCUUGCG------------------------------------------         24
------CACCCGUAGAACCuACCUUGCG------------------------------------------         27
------CACCCGUAGAACCGACCUUGC-------------------------------------------         28
------CACCCGUAGAACuGACCUUGCG------------------------------------------         30
------CACCCGUAGAACCGACCUUGCGa-----------------------------------------         155
------CACCCGUAGAACCGACCUUGCGu-----------------------------------------         293
------CACCCGUAGAACCGACCUUGCG------------------------------------------         4330

GGCACCCACCCGUAGAACCGACCUUGCGGGGCCUUCGCCGCACACAAGCUCGUGUCUGUGGGUCCGUGUC
(((((..(((((((((..(((.((((.(((((....))).).).)))).)))..)))))))))..)))))
```

hAgo2-top3   miR-142-3p   3P
hAgo2-top8   miR-142-5p   5P

```
-------------CCCAUAAAGUAGAAAGCACUACU---------------------------------------------------          13
-------------CCCAUAAAGUAGAAAGCACUACa---------------------------------------------------          17
-------------CCCAUAAAGUAGAAAGCACUACc---------------------------------------------------          34
-------------CCCAUAAAGUAGAAAGCACUA-----------------------------------------------------          120
-------------CCCAUAAAGUAGAAAGCACUAC----------------------------------------------------          1831
--------------CCAUAAAGUAGAAAGCACUAC----------------------------------------------------          23
---------------CAUAAAGUAGAAAGCACUACUA--------------------------------------------------          12
---------------CAUAAAGUAGAAAGCACUAC----------------------------------------------------          14
---------------CAUAAAGUAGAAAGCACUACU---------------------------------------------------          40
---------------------------------------------------UGUAGUGUUUCCUACUUUAUGGAa------------          12
---------------------------------------------------UGUAGUGUUUCCUACUUUAU----------------          17
---------------------------------------------------aGUAGUGUUUCCUACUUUAUGGA-------------          24
---------------------------------------------------UGUAGUGUUUCCUACUUUAUGG--------------          42
---------------------------------------------------UGUAGUGUUUCCUACUUUAUGGA-------------          373
----------------------------------------------------GUAGUGUUUCCUACUUUAUGGAU------------          10
----------------------------------------------------GcAGUGUUUCCUACUUUAUGGA-------------          11
----------------------------------------------------GUAGUGcUUCCUACUUUAUGGA-------------          13
----------------------------------------------------GUAGUGUUUCCUACUUUAUGGu-------------          17
----------------------------------------------------GUAGUGUUUCCUACUUUAUG---------------          22
----------------------------------------------------uUAGUGUUUCCUACUUUAUGGA-------------          34
----------------------------------------------------GUAGUGUUUCCUACUUUAUGGAa------------          65
----------------------------------------------------GUAGUGUUUCCUACUUUAUGG--------------          260
----------------------------------------------------GUAGUGUUUCCUACUUUAUGGA-------------          2952
-----------------------------------------------------cAGUGUUUCCUACUUUAUGGA-------------          19
-----------------------------------------------------UAGUGUUUCCUACUUUAUGGAU------------          27
-----------------------------------------------------UAGUGUUUCCUACUUUAUGGAa------------          42
-----------------------------------------------------UAGUGUUUCCUACUUUAUGGA-------------          104
------------------------------------------------------AGUGUUUCCUACUUUAUGGAU------------          16

GACAGUGCAGUCACCCAUAAAGUAGAAAGCACUACUAACAGCACUGGAGGGUGUAGUGUUUCCUACUUUAUGGAUGAGUGUACUGUG
.((((((((.(((.(((((((((((.(((((((((...(..(....)..)..))))))))).))))))))))).))).)))))))).
```

hAgo2-top4   miR-21   5P

```
------aUAGCUUAUCAGACUGAUGUUGA-------------------------------------------          10
-------UAGCUUAUCAGACUGAUGUUGACU-----------------------------------------          12
-------cAGCUUAUCAGACUGAUGUUGA-------------------------------------------          13
-------UAGCUUAcCAGACUGAUGUUGA-------------------------------------------          14
-------UAGCUUAUCAGACUGAUuUUGA-------------------------------------------          16
-------UAGCUUAUCAGACUGAUuUUGAC------------------------------------------          18
-------UAGCUUAUCAGACUGAUGUUGAa------------------------------------------          19
-------UAGCUUAUCAGACUGAUGUUGACa-----------------------------------------          58
-------UAGCUUAUCAGACUGAUGUUG--------------------------------------------          59
-------UAGCUUAUCAGACUGAUGUUGAC------------------------------------------          1544
-------UAGCUUAUCAGACUGAUGUUGA-------------------------------------------          2053
--------AGCUUAUCAGACUGAUGUUGAC------------------------------------------          29
--------AGCUUAUCAGACUGAUGUUGA-------------------------------------------          30
---------GCUUAUCAGACUGAUGUUGAC------------------------------------------          10
---------GCUUAUCAGACUGAUGUUGA-------------------------------------------          14
----------CUUAUCAGACUGAUGUUGA-------------------------------------------          16

UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACA
.(((((..((((((((.(((((.(((((.((((.(.....)))))))))).)))))))))))))..))))).
```

hAgo2-top5   **miR-92a-1**   **3P**

```
----------------------------------------------GUAUUGCACUUGUCCCGGCCUGU---------          13
-----------------------------------------------UAUUGCACUUGUCCCGcCCUG----------          10
-----------------------------------------------UAUUGuACUUGUCCCGGCCUGU---------          11
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGUU--------          12
-----------------------------------------------UAUUuCACUUGUCCCGGCCUGU---------          12
-----------------------------------------------UAUUGCACUUGUCCCGGCCUa----------          14
-----------------------------------------------UAUUGCACUUGUCCCGGCCUcU---------          17
-----------------------------------------------UAUUGCACUUGUCCCGGCCUaU---------          18
-----------------------------------------------UAUUGCACUUGUCCCGuCCUGU---------          24
-----------------------------------------------UAUUGCACUUuUCCCGGCCUGU---------          26
-----------------------------------------------UAUUGCACUUGUCCCGGCCU-----------          26
-----------------------------------------------UAUUGCACUcGUCCCGGCCU-----------          27
-----------------------------------------------UAUUGCACcUGUCCCGGCCUGU---------          27
-----------------------------------------------UAUUGCAuUUGUCCCGGCCUGU---------          36
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGUa--------          37
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGa---------          42
-----------------------------------------------UAUUGCACUUGUCCCGGCCUuU---------          72
-----------------------------------------------UAUUGCACUUGUCCCGGCCUG----------          160
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGU---------          1855
------------------------------------------------AUUGCACUUGUCCCGGCCUG----------          14
------------------------------------------------AUUGCACUUGUCCCGGCCUGU---------          151
-------------------------------------------------UUGCACUUGUCCCGGCCUGU---------          29
--------------------------------------------------UGCACUUGUCCCGGCCUGU---------          14

CUUUCUACACAGGUUGGGAUCGGUUGCAAUGCUGUGUUUCUGUAUGGUAUUGCACUUGUCCCGGCCUGUUGAGUUUGG
..(((...((((((((((((.(((.(((((((((((......)))))))))))))).))))))))))))).))).....
```

hAgo2-top5   miR-92a-2   3P

```
----------------------------------------------GUAUUGCACUUGUCCCGGCCUGU------    13
-----------------------------------------------UAUUGCACUUGUCCCGGcCCUG-------    10
-----------------------------------------------UAUUGuACUUGUCCCGGCCUGU------    11
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGUu-----    12
-----------------------------------------------UAUUuCACUUGUCCCGGCCUGU------    12
-----------------------------------------------UAUUGCACUUGUCCCGGCCUa-------    14
-----------------------------------------------UAUUGCACUUGUCCCGGCCUcU------    17
-----------------------------------------------UAUUGCACUUGUCCCGGCCUaU------    18
-----------------------------------------------UAUUGCACUUGUCCCGuCCUGU------    24
-----------------------------------------------UAUUGCACUUuUCCCGGCCUGU------    26
-----------------------------------------------UAUUGCACUUGUCCCGGCCU--------    26
-----------------------------------------------UAUUGCACUcGUCCCGGCCU--------    27
-----------------------------------------------UAUUGCACcUGUCCCGGCCUGU------    27
-----------------------------------------------UAUUGCAuUUGUCCCGGCCUGU------    36
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGUa-----    37
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGa------    42
-----------------------------------------------UAUUGCACUUGUCCCGGCCUuU------    72
-----------------------------------------------UAUUGCACUUGUCCCGGCCUG-------    160
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGU------    1855
------------------------------------------------AUUGCACUUGUCCCGGCCUG-------    14
------------------------------------------------AUUGCACUUGUCCCGGCCUGU------    151
-------------------------------------------------UUGCACUUGUCCCGGCCUGU------    29
--------------------------------------------------UGCACUUGUCCCGGCCUGU------    14

UCAUCCCUGGGUGGGGAUUUGUUGCAUUACUUGUGUUCUAUAUAAAGUAUUGCACUUGUCCCGGCCUGUGGAAGA
((.(((.(((((.(((((..((.(((.(((((((((...)))).))))).)))))..))))).))))).))).))
```

hAgo2-top6   miR-342-3p   3P

```
-----------------------------------------------------------aUCUCACACAGAAAUCGCACCCGU----------------      11
------------------------------------------------------------UCUCACACAGAAAUCGCACCCGUu---------------      10
------------------------------------------------------------UCUCACACAGAAAUCGCACCCGc----------------      11
------------------------------------------------------------UCUCACACAGAAAUCGCACCCGUa---------------      17
------------------------------------------------------------UCUCACACAGAAAUCGCACCCaU----------------      50
------------------------------------------------------------UCUCACACAGAAAUCGCACCCG-----------------      62
------------------------------------------------------------UCUCACACAGAAAUCGCACCCGUC---------------      669
------------------------------------------------------------UCUCACACAGAAAUCGCACCCGU----------------      1273
-------------------------------------------------------------CUCACACAGAAAUCGCACCCGUC---------------      26
-------------------------------------------------------------CUCACACAGAAAUCGCACCCGU----------------      35
--------------------------------------------------------------UCACACAGAAAUCGCACCCGUCA--------------      42
--------------------------------------------------------------UCACACAGAAAUCGCACCCGUC---------------      56

GAAACUGGGCUCAAGGUGAGGGGUGCUAUCUGUGAUUGAGGGACAUGGUUAAUGGAAUUGUCUCACACAGAAAUCGCACCCGUCACCUUGGCCUACUUA
.....(((((.((((((((.((((((..((((((.....((((((...((....))..)))))).)))))).....)))))).)))))))))))))....
```

hAgo2-top7 miR-484 5P

```
------aUCAGGCUCAGUCCCCUCCCGAU--------------------------------------------------         22
-------UCAGGCUCAGUCCCCUCCCGAUA-------------------------------------------------         14
-------UCAGGCUCAGUCCCCUCCCGAaA-------------------------------------------------         18
-------UCAGGCUCAuUCCCCUCCCGAU--------------------------------------------------         20
-------UCAGGCUCAGUCCuCUCCCGAU--------------------------------------------------         22
-------UCAGGCUCAGUCCCCUCCCGAa--------------------------------------------------         28
-------UCAGGCUCAGUCCCCUCCCGuU--------------------------------------------------         34
-------UCAGGCUCAGUCCCCUCCCGA---------------------------------------------------         73
-------UCAGGCUCAGUCCCCUCCCGAUu-------------------------------------------------        137
-------UCAGGCUCAGUCCCCUCCCGAU--------------------------------------------------       1573
--------CAGGCUCAGUCCCCUCCCGAUu-------------------------------------------------         14
--------CAGGCUCAGUCCCCUCCCGAU--------------------------------------------------        121
---------AGGCUCAGUCCCCUCCCGAU--------------------------------------------------         13

AGCCUCGUCAGGCUCAGUCCCCUCCCGAUAAACCCCUAAAUAGGGACUUUCCCGGGGGGUGACCCUGGCUUUUUUGGCG
.(((..((((((....(((.(((((((..(((.((((....))))..)))..))))))).)))))))))......))).
```

hAgo2-top9   miR-425   5P

```
------------aAAUGACACGAUCACUCCCGUUGA---------------------------------------------------       10
-------------AAUGACACGAUCACUCCCGUUGu---------------------------------------------------       10
-------------AAUGACACGAUCACUCCCuUUGA---------------------------------------------------       20
-------------AAUGACACGAUCACUCCCGUUGAG--------------------------------------------------       25
-------------AAUGACACGAUCACUCCCGUU-----------------------------------------------------       26
-------------AAUGACACuAUCACUCCCGUUGA---------------------------------------------------       38
-------------AAUGACACGAUCACUCCCGUUG----------------------------------------------------      386
-------------AAUGACACGAUCACUCCCGUUGA---------------------------------------------------      714
--------------AUGACACGAUCACUCCCGUUGAG--------------------------------------------------       13
--------------AUGACACGAUCACUCCCGUUGAGU-------------------------------------------------       18
--------------AUGACACGAUCACUCCCGUUG----------------------------------------------------       25
--------------AUGACACGAUCACUCCCGUUGA---------------------------------------------------       83
---------------UGACACGAUCACUCCCGUUGA---------------------------------------------------       11

GAAAGCGCUUUGGAAUGACACGAUCACUCCCGUUGAGUGGGCACCCGAGAAGCCAUCGGGAAUGUCGUGUCCGCCCAGUGCUCUUUC
(((((.((.((((...(((((((((((((.....)))))....(((((.......)))))...))))))))...)))).)).)))))
```

hAgo2-top10 miR-532-5p 5P

```
-------------------CAUGCCUUGAGUGUAuGACCGU--------------------------------------------------      11
-------------------CAUGCCUUGAGUuUAGGACCGU--------------------------------------------------      11
-------------------CAUGCCUUGAGUGUAGGACC----------------------------------------------------      12
-------------------CAUGCCUUGAGUGUAGGACCGUU-------------------------------------------------      14
-------------------CAUGCCUUGAGUGUAGuACCGU--------------------------------------------------      15
-------------------CAUGCCUUGAGUGUAGGACCGa--------------------------------------------------      17
-------------------CAUGCCUUGAGUGUAGGACCGUa-------------------------------------------------      22
-------------------CAUGCCUUGAGUGUAGGACCG---------------------------------------------------      32
-------------------CAUGCCUUGAGUGUAGGACCGU--------------------------------------------------      1036
--------------------AUGCCUUGAGUGUAGGACCGU--------------------------------------------------      65
---------------------UGCCUUGAGUGUAGGACCGU--------------------------------------------------      14

CGACUUGCUUUCUCUCCUCCAUGCCUUGAGUGUAGGACCGUUGGCAUCUUAAUUACCCUCCCACACCCAAGGCUUGCAAAAAAGCGAGCCU
.(.((((((((........((.((((((.((((.(((..((.............))..))).)))).)))))).))....)))))))).).
```

hAgo2-top11   miR-17*   3P

```
-------------------------------------------------aACUGCAGUGAAGGCACUUGUAGC-----------    10
--------------------------------------------------ACUGCAGUcAAGGCACUUGUAG------------    10
--------------------------------------------------ACUGCAGUGAAGGCACUUGUAG------------   232
--------------------------------------------------ACUGCAGUGAAGGCACUUGUAGC-----------   423
---------------------------------------------------CUGCAGUGAAGGCACUUGUAG------------    86
---------------------------------------------------CUGCAGUGAAGGCACUUGUAGC-----------   139
----------------------------------------------------UGCAGUGAAGGCACUUGUAG------------    12
----------------------------------------------------UGCAGUGAAGGCACUUGUAGC-----------    64

GUCAGAAUAAUGUCAAAGUGCUUACAGUGCAGGUAGUGAUAUGUGCAUCUACUGCAGUGAAGGCACUUGUAGCAUUAUGGUGAC
((((..(((((((..((((((((.((.(((((.(((((.......)).)))))))).)).))))))))...)))))))..))))
```

hAgo2-top12 miR-210 3P

```
-----------------------------------------------------------------CUGUGCGUGUGACAGCGaCUGA-----------------------    11
-----------------------------------------------------------------CUGUGCGUGUGACAuCGGCUGA-----------------------    13
-----------------------------------------------------------------CUGUGCGUGUGACAGCGGCUGu-----------------------    14
-----------------------------------------------------------------CUGUGCGUGUGACAGCGGCUGAa----------------------    15
-----------------------------------------------------------------CUGUGCGUGUGACAGCGGCUGAU----------------------    22
-----------------------------------------------------------------CUGUGCGUGUGACAGCGGCUGA-----------------------    721
------------------------------------------------------------------UGUGCGUGUGACAGCGGCUGA-----------------------    15
-------------------------------------------------------------------GUGCGUGUGACAGCGGCUGA-----------------------    13
ACCCGGCAGUGCCUCCAGGCGCAGGGCAGCCCCUGCCCACCGCACACUGCGCUGCCCCAGACCCACUGUGCGUGUGACAGCGGCUGAUCUGUGCCUGGGCAGCGCGACCC
....((..((((.(((((((((((..(((((.(((..(((((((((.((.((((...))).).)).))))))).))).))).))))..)))))))))))..))))..)).
```

hAgo2-top13   miR-16-1   5P

```
-------------UAGCAGCACGUAAAUAUUGGCGU-----------------------------------------------------        51
-------------UAGCAGCACGUAAAUAUUGGCG------------------------------------------------------        641

GUCAGCAGUGCCUUAGCAGCACGUAAAUAUUGGCGUUAAGAUUCUAAAAUUAUCUCCAGUAUUAACUGUGCUGCUGAAGUAAGGUUGAC
((((((..(((.((((((((((((.((((((((.(.(((..........))).).)))))))).)).))))))))))).)))..))))))
```

hAgo2-top13   miR-16-2   5P

```
---------UAGCAGCACGUAAAUAUUGGCGU-------------------------------------------------        51
---------UAGCAGCACGUAAAUAUUGGCG--------------------------------------------------        641

GUUCCACUCUAGCAGCACGUAAAUAUUGGCGUAGUGAAAUAUAUAUUAAACACCAAUAUUACUGUGCUGCUUUAGUGUGAC
((..((((..(((((((((..((((((((.((..(((........))).)).))))))))..)))))))))..))))..))
```

hAgo2-top14   miR-25   3P

```
---------------------------------------------------CAUUGCACUUGUCUCGGUCUGAa----------    11
---------------------------------------------------uAUUGCACUUGUCUCGGUCUGA-----------    20
---------------------------------------------------CAUUGCACUUGUCUCGGUCUGA-----------    605

GGCCAGUGUUGAGAGGCGGAGACUUGGGCAAUUGCUGGACGCUGCCCUGGGCAUUGCACUUGUCUCGGUCUGACAGUGCCGGCC
((((.(..(((..((((.(((((..(.(((((.(((((.......))..)))))))).)..))))).))))..)))..).))))
```

hAgo2-top15   miR-423-3p   3P

```
---------------------------------------------------AAGCUCGGUCUGAGGCCCCUCAGU-------------------           24
----------------------------------------------------AGCUCGGUCUGAGGCCCCUCAuU-------------------           10
----------------------------------------------------AGCUCGGUCUGAuGCCCCUCAGU-------------------           10
----------------------------------------------------AGCUCGGUCUGAGGCCCCUCAGUu------------------           10
----------------------------------------------------AGCUCGGUCUGAGGCCCCUCAG--------------------           54
----------------------------------------------------AGCUCGGUCUGAGGCCCCUCAGU-------------------           439
-----------------------------------------------------GCUCGGUCUGAGGCCCCUCAGU-------------------           31
------------------------------------------------------CUCGGUCUGAGGCCCCUCAGU-------------------           41

AUAAAGGAAGUUAGGCUGAGGGGCAGAGAGCGAGACUUUUCUAUUUUCCAAAAGCUCGGUCUGAGGCCCCUCAGUCUUGCUUCCUAACCCGCGC
....(((((((.((((((((((((..(((.((((.(((((.........))))))))).)))...)))))))))))).))))))).........
```

hAgo2-top16 miR-92b 3P

```
----------------------------------------------------------UAUUGCACUCGUCCCGGCCUCCu-------------          10
----------------------------------------------------------UAUUGCACUCGUCCCGGCCUu---------------          13
----------------------------------------------------------UAUUGCACUCGUCCCGGCCUCu--------------          16
----------------------------------------------------------UAUUGCACUuGUCCCGGCCU----------------          26
----------------------------------------------------------UAUUGCACUCGUCCCGGCCU----------------          27
----------------------------------------------------------UAUUGCACUCGUCCCGGCCUCa--------------          29
----------------------------------------------------------UAUUGCACUCGUCCCGGCCUC---------------         140
----------------------------------------------------------UAUUGCACUCGUCCCGGCCUCC--------------         314

CGGGCCCCGGGCGGGCGGAGGGACGGGACGCGGUGCAGUGUUGUUUUUCCCCCGCCAAUAUUGCACUCGUCCCGGCCUCCGGCCCCCCCGGCCC
.(((((..(((.((((.(((((..(((((((.((((((((((((...........))))))))))))))))))).))))).))))))).)))))
```

hAgo2-top17 let-7d* 3P

```
-------------------------------------------------------------CUAUACGACCUGCUGCCUUUCa----          14
-------------------------------------------------------------CUAUACGACCUGCUGCCUUUC-----          50
-------------------------------------------------------------CUAUACGACCUGCUGCCUUUCU----          396
--------------------------------------------------------------UAUACGACCUGCUGCCUUUCU----          96
---------------------------------------------------------------AUACGACCUGCUGCCUUUCU----          21

CCUAGGAAGAGGUAGUAGGUUGCAUAGUUUUAGGGCAGGGAUUUUGCCCACAAGGAGGUAACUAUACGACCUGCUGCCUUUCUUAGG
(((((((.((((((((((((((.((((((...((((((.....)))))).(.....)..)))))).)))))))))))))))))))))
```

hAgo2-top18   miR-191   5P

```
---------------CAACGuAAUCCCAAAAGCAGCUG------------------------------------------------------     11
---------------CAACGGAAUCCCAAAAGCAGC-------------------------------------------------------     22
---------------CAACGGAAUCCCAAAAGCAGCUGU-----------------------------------------------------     26
---------------CAACGGAAUCCCAAAAGCAGCUG------------------------------------------------------    172
---------------CAACGGAAUCCCAAAAGCAGCU-------------------------------------------------------    264

CGGCUGGACAGCGGGCAACGGAAUCCCAAAAGCAGCUGUUGUCUCCAGAGCAUUCCAGCUGCGCUUGGAUUUCGUCCCCUGCUCUCCUGCCU
.(((.(((.((((((..(((((((((.((..(((((((..((((....)).))..)))))))..)))))))))))..)))))).))).))).
```

hAgo2-top19 miR-128-1 3P

```
-------------------------------------------------UCACAGUGAACCGGUCUCUUUa-----------     10
-------------------------------------------------UCACAGUGAACCGGUCUCUUUU-----------     61
-------------------------------------------------UCACAGUGAACCGGUCUCUUU------------     442

UGAGCUGUUGGAUUCGGGGCCGUAGCACUGUCUGAGAGGUUUACAUUUCUCACAGUGAACCGGUCUCUUUUUCAGCUGCUUC
.((((.((((((...(((((((...((((((..(((((((....)))))))))))))...)))))))...)))))).)))).
```

hAgo2-top19 miR-128-2 3P

```
---------------------------------------------------UCACAGUGAACCGGUCUCUUUa-----------     10
---------------------------------------------------UCACAGUGAACCGGUCUCUUUu-----------     61
---------------------------------------------------UCACAGUGAACCGGUCUCUUU------------     442

UGUGCAGUGGGAAGGGGGGCCGAUACACUGUACGAGAGUGAGUAGCAGGUCUCACAGUGAACCGGUCUCUUUCCCUACUGUGUC
.(..(((((((.((((((((((...((((((..((((.(........).))))))))))...)))))))))).)))))))..).
```

hAgo2-top20   miR-28-3p   3P

```
-----------------------------------------------------CACUAGAUUGUGAGCUCCUGGAa----------      25
-----------------------------------------------------CACUAGAUUGUGAGCUCCUGG------------      62
-----------------------------------------------------CACUAGAUUGUGAGCUCCUGGA-----------      311

GGUCCUUGCCCUCAAGGAGCUCACAGUCUAUUGAGUUACCUUUCUGACUUUCCCACUAGAUUGUGAGCUCCUGGAGGGCAGGCACU
(((.(((((((((.((((((((((((((((.(((((((......)))))....)).)))))))))))))))).))))))))).)))
```

hAgo2-top21 miR-221 3P

```
----------------------------------------------------------------AGCUACAUUGUCUGCUGGGUU-------------------------    15
----------------------------------------------------------------AGCUACAUUGUCUGCUGGGUUUu-----------------------    15
----------------------------------------------------------------AGCUACAUUGUCUGCUGGGUUUC-----------------------    91
----------------------------------------------------------------AGCUACAUUGUCUGCUGGGUUU------------------------    254
-----------------------------------------------------------------GCUACAUUGUCUGCUGGGUUU------------------------    15

UGAACAUCCAGGUCUGGGGCAUGAACCUGGCAUACAAUGUAGAUUUCUGUGUUCGUUAGGCAACAGCUACAUUGUCUGCUGGGUUUCAGGCUACCUGGAAACAUGUUCUC
.((((((((((((....(((.((((((..(((.(((((((((....(((((((.....))).))))))))))))).)))..)).)))).))))))))))....)))))..
```

hAgo2-top22 miR-192 5P

```
-----------------------CUGACCUAUGAAUUGACAGCCAG----------------------------------------------------------------   11
-----------------------CUGACCUAUGAAUUGACAGCCAu----------------------------------------------------------------   14
-----------------------CUGACCUAUGAAUUGACAGCCA-----------------------------------------------------------------   71
-----------------------CUGACCUAUGAAUUGACAGCC------------------------------------------------------------------   110
------------------------UGACCUAUGAAUUGACAGCCAGU---------------------------------------------------------------   15
------------------------UGACCUAUGAAUUGACAGCCA-----------------------------------------------------------------   21
------------------------UGACCUAUGAAUUGACAGCCAG----------------------------------------------------------------   104

GCCGAGACCGAGUGCACAGGGCUCUGACCUAUGAAUUGACAGCCAGUGCUCUCGUCUCCCCUCUGGCUGCCAAUUCCAUAGGUCACAGGUAUGUUCGCCUCAAUGCCAGC
.........(((.(((((.(.((.((((((((((((((.(((((((................))))))).))))).))))))))).)).).))).)))..))))).........
```

hAgo2-top23 miR-15a 5P

```
-------------UAGCAGCACAUAAUGGUUUGUGa----------------------------------------------      15
-------------UAGCAGCACAUAAUGGUUUGU------------------------------------------------      91
-------------UAGCAGCACAUAAUGGUUUGUG-----------------------------------------------      233

CCUUGGAGUAAAGUAGCAGCACAUAAUGGUUUGUGGAUUUGAAAAGGUGCAGGCCAUAUUGUGCUGCCUCAAAAAUACAAGG
(((((.......(..((((((((..((((((((((..((.....))..))))))))))..))))))))..).......)))))
```

hAgo2-top24   miR-941-1   3P

```
------------------------------------------------------CACCCGGCUGUGUGCACAUGUG-------------        31
------------------------------------------------------CACCCGGCUGUGUGCACAUGUGC------------        309

CCCGGCUGUGUGGACAUGUGCCCAGGGCCCGGGACAGCGCCACGGAAGAGGACGCACCCGGCUGUGUGCACAUGUGCCCAGGGCCCGGG
(((((((.((.((((((((((.((.((((.(((...(((((.(....).)).))).))))))).)).)))))))).)))).)).)))))
```

hAgo2-top24   miR-941-2   3P

```
------------------------------------------------------CACCCGGCUGUGUGCACAUGUG-------------        31
------------------------------------------------------CACCCGGCUGUGUGCACAUGUGC------------        309

CCCGGCUGUGUGCACAUGUGCCCAGGGCCCGGGACAGCGCCACGGAAGAGGACGCACCCGGCUGUGUGCACAUGUGCCCAGGGCCCGGG
(((((((.((.((((((((((.((.((((.(((...(((((.(....).)).))).))))))).)).)))))))))).)).)).)))))
```

hAgo2-top25 miR-197 3P

```
-----------------------------------------------UUCACCACCUUCUCCACCCAG-------    11
-----------------------------------------------UUCACCACCUUCUCCACCCAGCA-----    18
-----------------------------------------------UUCACCACCUUCUCCACCCAGu------    34
-----------------------------------------------UUCACCACCUUCUCCACCCAGC------    243

GGCUGUGCCGGGUAGAGAGGGCAGUGGGAGGUAAGAGCUCUUCACCCUUCACCACCUUCUCCACCCAGCAUGGCC
((((((((.((((.(((((((..((((..((((((....))).)))..))))..))))))).)))).))))))))
```

hAgo2-top26 miR-29c 3P

```
-----------------------------------------------------UAGCACCAUUUGAAAUCGGUU--------------            18
-----------------------------------------------------UAGCACCAUcUGAAAUCGGUUA-------------            74
-----------------------------------------------------UAGCACCAUUUGAAAUCGGUUA-------------            301

AUCUCUUACACAGGCUGACCGAUUUCUCCUGGUGUUCAGAGUCUGUUUUUGUCUAGCACCAUUUGAAAUCGGUUAUGAUGUAGGGGGA
.((((((((((...(((((((((((...(((((((((((((.....)))))...)))))))...))))))))))))).))))))))).
```

hAgo2-top27 miR-766 3P

```
----------------------------------------------------------------ACUCCAGCCCCACAGCCUCAGa-------------------------   16
----------------------------------------------------------------ACUCCAGCCCCACAGCCUCAG--------------------------   41
----------------------------------------------------------------ACUCCAGCCCCACAGCCUCAGu-------------------------   73
----------------------------------------------------------------ACUCCAGCCCCACAGCCUCAGC-------------------------   173

GCAUCCUCAGGACCUGGGCUUGGGUGGUAGGAGGAAUUGGUGCUGGUCUUUCAUUUUGGAUUUGACUCCAGCCCCACAGCCUCAGCCACCCCAGCCAAUUGUCAUAGGAGC
((.((((...(((...((((.(((((((..((((...(((.(((((....(((.........)))..))))).)))...)))).))))))).))))....)))..))))))
```

hAgo2-top28 miR-345 5P

```
-----------------GCUGACUCCUAGUCCAGGGCUCu----------------------------------------------------------       13
-----------------GCUGACUCCUAGUCCAGGGCUu-----------------------------------------------------------       31
-----------------GCUGACUCCUAGUCCAGGGCU------------------------------------------------------------       72
-----------------GCUGACUCCUAGUCCAGGGCUC-----------------------------------------------------------       134
------------------CUGACUCCUAGUCCAGGGCUC-----------------------------------------------------------       24

ACCCAAACCCUAGGUCUGCUGACUCCUAGUCCAGGGCUCGUGAUGGCUGGUGGGCCCUGAACGAGGGGUCUGGAGGCCUGGGUUUGAAUAUCGACAGC
...(((((((.((((((.(.(((((((.((.(((((((((..(....)..))))))))).)).))))))).).)))))))))))))............
```

hAgo2-top29 miR-93* 3P

```
-------------------------------------------------ACUGCUGAGCUAGCACUUCCCGu--------        33
-------------------------------------------------ACUGCUGAGCUAGCACUUCCCG---------        106
-------------------------------------------------ACUGCUGAGCUAGCACUUCCCGA--------        122

CUGGGGGCUCCAAAGUGCUGUUCGUGCAGGUAGUGUGAUUACCCAACCUACUGCUGAGCUAGCACUUCCCGAGCCCCCGG
((((((((((..((((((((((((.((((.(((..((......))..))))))))))))).)))))))...))))))))))
```

hAgo2-top30   miR-501-3p   3P

```
--------------------------------------------------AAUGCACCCGGGCAAuGAUUCU------------   10
--------------------------------------------------AAUGCACCCGGGCAAGGAUUCU------------   199
---------------------------------------------------AUGCACCCGGGCAAGGAUUCU------------   19

GCUCUUCCUCUCUAAUCCUUUGUCCCUGGGUGAGAGUGCUUUCUGAAUGCAAUGCACCCGGGCAAGGAUUCUGAGAGGGUGAGC
((((.(((((((.(((((((.(.(((.(((((....(((.........)))...))))))))))))))))..))))))).))))
```

hAgo3-top1 **miR-142-3p** **3P**
hAgo3-top16 **miR-142-5p** **5P**

```
-------------CCCAUAAAGUAGAAAGCACUACc---------------------------------------------------          18
-------------CCCAUAAAGUAGAAAGCACUA-----------------------------------------------------          46
-------------CCCAUAAAGUAGAAAGCACUAC----------------------------------------------------          181
---------------CAUAAAGUAGAAAGCACUACU---------------------------------------------------          37
---------------CAUAAAGUAGAAAGCACUACUA--------------------------------------------------          227
---------------------------------------------------UGUAGUGUUUCCUACUUUAUGGA-------------          386
---------------------------------------------------UGUAGUGUUUCCUACUUUAUGG--------------          651
----------------------------------------------------GUAGUGUUUCCUACUUUAUGGu-------------          19
----------------------------------------------------uUAGUGUUUCCUACUUUAUGGA-------------          37
----------------------------------------------------GUAGUGUUUCCUACUUUAUGGAa------------          42
----------------------------------------------------GUAGUGUUUCCUACUUUAUG---------------          86
----------------------------------------------------GUAGUGUUUCCUACUUUAUGGA-------------          3685
----------------------------------------------------GUAGUGUUUCCUACUUUAUGG--------------          12636
-----------------------------------------------------UAGUGUUUCCUACUUUAUGGA-------------          20

GACAGUGCAGUCACCCAUAAAGUAGAAAGCACUACUAACAGCACUGGAGGGUGUAGUGUUUCCUACUUUAUGGAUGAGUGUACUGUG
.((((((((.(((.(((((((((((.(((((((((...(..(....)..)..))))))))).))))))))))).))).)))))))).
```

hAgo3-top2   miR-150   5P

```
-------------UGUCUCCCAACCCUUGUACCAGU------------------------------------------------    10
--------------aUCUCCCAACCCUUGUACCAGUG-----------------------------------------------    14
---------------UCUCCCAACCCUUGUACCAGUu-----------------------------------------------    13
---------------UCUCCCAACCCUUGcACCAGUG-----------------------------------------------    16
---------------UCUCCCAACCCUcGUACCAGUG-----------------------------------------------    23
---------------UCUCCCAACCCUUuUACCAGUG-----------------------------------------------    30
---------------UCUCCCAACCCUUGUACCAGUa-----------------------------------------------    71
---------------UCUCCCAACCCUUGUACCAGUGC----------------------------------------------    263
---------------UCUCCCAACCCUUGUACCAGU------------------------------------------------    286
---------------UCUCCCAACCCUUGUACCAGUGa----------------------------------------------    663
---------------UCUCCCAACCCUUGUACCAGUGu----------------------------------------------    755
---------------UCUCCCAACCCUUGUACCAGUG-----------------------------------------------    9951
----------------CUCCCAACCCUUGUACCAGUGu----------------------------------------------    13
----------------CUCCCAACCCUUGUACCAGUG-----------------------------------------------    52

CUCCCCAUGGCCCUGUCUCCCAACCCUUGUACCAGUGCUGGGCUCAGACCCUGGUACAGGCCUGGGGGACAGGGACCUGGGGAC
.((((((.(((((((((((((..(((.((((((((.((((....))).).))))))))))..)))))))))))).)))))))).
```

hAgo3-top3   miR-92b   3P

```
------------------------------------------------------------UAUUGCACUCGUCCCGGCCU----------------          16
------------------------------------------------------------UAUUGCACUuGUCCCGGCCU----------------          20
------------------------------------------------------------UAUUGCACUCGUCCCGGCCUu---------------          45
------------------------------------------------------------UAUUGCACUCGUCCCGGCCUCu--------------          211
------------------------------------------------------------UAUUGCACUCGUCCCGGCCUCCu-------------          335
------------------------------------------------------------UAUUGCACUCGUCCCGGCCUCa--------------          439
------------------------------------------------------------UAUUGCACUCGUCCCGGCCUC---------------          1292
------------------------------------------------------------UAUUGCACUCGUCCCGGCCUCC--------------          4835

CGGGCCCCGGGCGGGCGGGAGGGACGGGACGCGGUGCAGUGUUGUUUUUUCCCCCGCCAAUAUUGCACUCGUCCCGGCCUCCGGCCCCCCCGGCCC
.(((((..(((.((((.(((((..(((((((.((((((((((((.............))))))))))))))))))).))))).))))))).)))))
```

hAgo3-top4 miR-425 5P

```
------------aAAUGACACGAUCACUCCCGUUGA---------------------------------------------------          33
-------------AAUGACACGAUCACUCCCuUUGA---------------------------------------------------          11
-------------AAUGACACGAUCACUCCCGUUG----------------------------------------------------          97
-------------AAUGACACuAUCACUCCCGUUGA---------------------------------------------------          105
-------------AAUGACACGAUCACUCCCGUUGA---------------------------------------------------          4070
--------------AUGACACGAUCACUCCCGUUGA---------------------------------------------------          180
---------------UGACACGAUCACUCCCGUUGA---------------------------------------------------          10

GAAAGCGCUUUGGAAUGACACGAUCACUCCCGUUGAGUGGGCACCCGAGAAGCCAUCGGGAAUGUCGUGUCCGCCCAGUGCUCUUUC
(((((.((.((((...(((((((((((((.....)))))....(((((.......)))))...))))))))...)))).)).)))))
```

hAgo3-top5 **miR-532-5p** **5P**

```
-------------------CAUGCCUUGAGUGUAGGACCGUU-------------------------------------------------     14
-------------------CAUGCCUUGAGUGUAGuACCGU--------------------------------------------------     17
-------------------CAUGCCUUGAGUGUAuGACCGU--------------------------------------------------     17
-------------------CAUGCCUUGAGUGUAGGACCGa--------------------------------------------------     32
-------------------CAUGCCUUGAGUGUAGGACC----------------------------------------------------     66
-------------------CAUGCCUUGAGUGUAGGACCGUa-------------------------------------------------     92
-------------------CAUGCCUUGAGUGUAGGACCG---------------------------------------------------    395
-------------------CAUGCCUUGAGUGUAGGACCGU--------------------------------------------------   3653
--------------------AUGCCUUGAGUGUAGGACCGU--------------------------------------------------    177

CGACUUGCUUUCUCUCCUCCAUGCCUUGAGUGUAGGACCGUUGGCAUCUUAAUUACCCUCCCACACCCAAGGCUUGCAAAAAAGCGAGCCU
.(.((((((((........((.((((((.((((.(((..((.............))..))).)))).)))))).))....)))))))).).
```

hAgo3-top6   miR-92a-1   3P

```
-----------------------------------------------UAUUGCACUUGUCCCGGCCUa----------          15
-----------------------------------------------UAUUGCACUcGUCCCGGCCU-----------          16
-----------------------------------------------UAUUGCACUUGUCCCGGCCUcU---------          16
-----------------------------------------------UAUUGCACUUGUCCCGGCCU-----------          20
-----------------------------------------------UAUUGCAuUUGUCCCGGCCUGU---------          24
-----------------------------------------------UAUUGCACUUuUCCCGGCCUGU---------          30
-----------------------------------------------UAUUGCACUUGUCCCGGCCUuU---------          35
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGUU--------          47
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGUa--------          65
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGa---------          67
-----------------------------------------------UAUUGCACUUGUCCCGGCCUG----------          280
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGU---------          2171
------------------------------------------------AUUGCACUUGUCCCGGCCUGU---------          53

CUUUCUACACAGGUUGGGAUCGGUUGCAAUGCUGUGUUUCUGUAUGGUAUUGCACUUGUCCCGGCCUGUUGAGUUUGG
..(((...((((((((((((.(((.(((((((((((......))))))))))))))).))))))))))))).))).....
```

hAgo3-top6 miR-92a-2 3P

```
-----------------------------------------------UAUUGCACUUGUCCCGGCCUa-------    15
-----------------------------------------------UAUUGCACUcGUCCCGGCCU--------    16
-----------------------------------------------UAUUGCACUUGUCCCGGCCUcU------    16
-----------------------------------------------UAUUGCACUUGUCCCGGCCU--------    20
-----------------------------------------------UAUUGCAuUUGUCCCGGCCUGU------    24
-----------------------------------------------UAUUGCACUUuUCCCGGCCUGU------    30
-----------------------------------------------UAUUGCACUUGUCCCGGCCUuU------    35
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGUu-----    47
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGUa-----    65
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGa------    67
-----------------------------------------------UAUUGCACUUGUCCCGGCCUG-------    280
-----------------------------------------------UAUUGCACUUGUCCCGGCCUGU------    2171
------------------------------------------------AUUGCACUUGUCCCGGCCUGU------    53

UCAUCCCUGGGUGGGGAUUUGUUGCAUUACUUGUGUUCUAUAUAAAGUAUUGCACUUGUCCCGGCCUGUGGAAGA
((.(((.(((((.(((((..((.(((.(((((((((...)))).))))).)))))..))))).))))).))).))
```

hAgo3-top7   **miR-629***   **3P**

```
------------------------------------------------------------aUUCUCCCAACGUAAGCCCAGC---------------        358
------------------------------------------------------------GUUCUCCCAACGUAAGCCCAGC---------------        1318
-------------------------------------------------------------UUCUCCCAACGUAAGCCCAGC---------------        51

UCCCUUUCCCAGGGGAGGGGCUGGGUUUACGUUGGGAGAACUUUUACGGUGAACCAGGAGGUUCUCCCAACGUAAGCCCAGCCCCUCCCCUCUGCCU
..........(((((((((((((((((((((((((((((((((((..((....)).)))))))))))))))))))))))))))))))))))......
```

hAgo3-top8 miR-210 3P

```
-----------------------------------------------------------------CUGUGCGUGUGACAGCGGCUGu-----------------------    17
-----------------------------------------------------------------CUGUGCGUGUGACAuCGGCUGA-----------------------    19
-----------------------------------------------------------------CUGUGCGUGUGACAGCGGCUGAa----------------------    31
-----------------------------------------------------------------CUGUGCGUGUGACAGCGGCUGAU----------------------    37
-----------------------------------------------------------------CUGUGCGUGUGACAGCGGCUGA-----------------------    2561

ACCCGGCAGUGCCUCCAGGCGCAGGGCAGCCCCUGCCCACCGCACACUGCGCUGCCCCAGACCCACUGUGCGUGUGACAGCGGCUGAUCUGUGCCUGGGCAGCGCGACCC
....((..((((.(((((((((((..(((((.(((..(((((((((.((.((((...))).).)).)))))).))).))).)))))..)))))))))))..))))..)).
```

hAgo3-top9   miR-484   5P

```
-------UCAGGCUCAGUCCCCUCCCGA--------------------------------------------------        57
-------UCAGGCUCAGUCCCCUCCCGuU-------------------------------------------------        59
-------UCAGGCUCAGUCCCCUCCCGAaA------------------------------------------------        72
-------UCAGGCUCAGUCCCCUCCCGAa-------------------------------------------------        81
-------UCAGGCUCAGUCCCCUCCCGAUA------------------------------------------------       142
-------UCAGGCUCAGUCCCCUCCCGAUu------------------------------------------------       605
-------UCAGGCUCAGUCCCCUCCCGAU-------------------------------------------------       813

AGCCUCGUCAGGCUCAGUCCCCUCCCGAUAAACCCCUAAAUAGGGACUUUCCCGGGGGUGACCCUGGCUUUUUUGGCG
.(((..((((((....(((.(((((((..(((.((((....))))..)))..))))))).)))))))))......))).
```

hAgo3-top10 miR-191 5P

```
---------------CAACGGAAUCCCAAAAGCAGC--------------------------------------------------------    132
---------------CAACGGAAUCCCAAAAGCAGCUG------------------------------------------------------    145
---------------CAACGGAAUCCCAAAAGCAGCU-------------------------------------------------------    1273

CGGCUGGACAGCGGGCAACGGAAUCCCAAAAGCAGCUGUUGUCUCCAGAGCAUUCCAGCUGCGCUUGGAUUUCGUCCCCUGCUCUCCUGCCU
.(((.(((.((((((..(((((((((.((..(((((((..((((....)).))..)))))))..)))))))))))..)))))).))).))).
```

hAgo3-top11 miR-21 5P

```
-------UAGCUUAUCAGACUGAUGUUGAa------------------------------------------       31
-------UAGCUUAUCAGACUGAUGUUG--------------------------------------------       55
-------UAGCUUAUCAGACUGAUGUUGAC------------------------------------------       62
-------UAGCUUAUCAGACUGAUGUUGA-------------------------------------------       1191

UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACA
.(((((..((((((((.(((((.(((((.((((.(.....)))))))))).)))))))))))))..))))).
```

hAgo3-top12 miR-99b 5P

```
------CACCCGUAGAACCGACCUUGC-------------------------------------------         17
------CACCCGUAGAACCGACCUUGCGa-----------------------------------------         20
------CACCCGUAGAACCGACCUUGCG------------------------------------------         717

GGCACCCACCCGUAGAACCGACCUUGCGGGGCCUUCGCCGCACACAAGCUCGUGUCUGUGGGUCCGUGUC
(((((..(((((((((..(((.((((.(((((....))).).).)))).)))..)))))))))..)))))
```

hAgo3-top13 miR-93* 3P

```
-------------------------------------------------ACUGCUGAGCUAGCACUUCCCGu--------          35
-------------------------------------------------ACUGCUGAGCUAGCACUUCCCG---------          265
-------------------------------------------------ACUGCUGAGCUAGCACUUCCCGA--------          367

CUGGGGGCUCCAAAGUGCUGUUCGUGCAGGUAGUGUGAUUACCCAACCUACUGCUGAGCUAGCACUUCCCGAGCCCCCGG
((((((((((..((((((((((((.((((.(((..((......))..)))))))))))))).)))))))...))))))))))
```

hAgo3-top14 **miR-222** **3P**

```
--------------------------------------------------------------------AGCUACAUCUGGCUACUGGGUCUC------------------    52
--------------------------------------------------------------------AGCUACAUCUGGCUACUGGGUCU-------------------    237
--------------------------------------------------------------------AGCUACAUCUGGCUACUGGGUC--------------------    332
---------------------------------------------------------------------GCUACAUCUGGCUACUGGGUCU-------------------    15
---------------------------------------------------------------------GCUACAUCUGGCUACUGGGUC--------------------    17

GCUGCUGGAAGGUGUAGGUACCCUCAAUGGCUCAGUAGCCAGUGUAGAUCCUGUCUUUCGUAAUCAGCAGCUACAUCUGGCUACUGGGUCUCUGAUGGCAUCUUCUAGCU
...(((((((((((......((.(((..(((((((((((((((((((...(((...........)))...))))).))))))))))))))..))).))))))))))))).
```

hAgo3-top15 let-7d* 3P
hAgo3-top28 let-7d 5P

```
-------AGAGGUAGUAGGUUGCAUAGU-----------------------------------------------------------          15
-------AGAGGUAGUAGGUUGCAUAGUU----------------------------------------------------------          166
-------------------------------------------------------------CUAUACGACCUGCUGCCUUUCa----          14
-------------------------------------------------------------CUAUACGACCUGCUGCCUUUC-----          42
-------------------------------------------------------------CUAUACGACCUGCUGCCUUUCU----          539

CCUAGGAAGAGGUAGUAGGUUGCAUAGUUUUAGGGCAGGGAUUUUGCCCACAAGGAGGUAACUAUACGACCUGCUGCCUUUCUUAGG
((((((.(((((((((((((((.((((((...((((((.....)))))).(.....)..)))))).)))))))))))))))))))))
```

hAgo3-top17　　miR-25　　3P

```
---------------------------------------------------CAUUGCACUUGUCUCGGUCUGA-----------     429

GGCCAGUGUUGAGAGGCGGAGACUUGGGCAAUUGCUGGACGCUGCCCUGGGCAUUGCACUUGUCUCGGUCUGACAGUGCCGGCC
((((.(..(((..((((.(((((..(.(((((.(((((.......))..)))))))).)..))))).))))..)))..).))))
```

hAgo3-top18 miR-26a 5P

```
---------UUCAAGUAAUCCAGGAUAGG------------------------------------------------     48
---------UUCAAGUAAUCCAGGAUAGGC-----------------------------------------------    107
---------UUCAAGUAAUCCAGGAUAGGCU----------------------------------------------    222

GUGGCCUCGUUCAAGUAAUCCAGGAUAGGCUGUGCAGGUCCCAAUGGGCCUAUUCUUGGUUACUUGCACGGGGACGC
(((.((((((.(((((((((.((((((((((.((.......))...)))))))))).))))))))).)))))).)))
```

hAgo3-top19 miR-324-5p 5P

```
---------------CGCAUCCCCUAGGGCAUUGGUGU---------------------------------------------   14

CUGACUAUGCCUCCCCGCAUCCCCUAGGGCAUUGGUGUAAAGCUGGAGACCCACUGCCCCAGGUGCUGCUGGGGGUUGUAGUC
..((((((..(((((.(((.(.(((.(((((.(((.((..........))))).))))).))).).))).))))).))))))
```

hAgo3-top20　　miR-23a　　3P

```
--------------------------------------------AUCACAUUGCCAGGGAUUUCC--------     13
--------------------------------------------AUCACAUUGCCAGGGAUUaCCA-------     29
--------------------------------------------AUCACAUUGCCAGGGAUUUCCAA------     72
--------------------------------------------AUCACAUUGCCAGGGAUUUCCA-------     265

GGCCGGCUGGGGUUCCUGGGGAUGGGAUUUGCUUCCUGUCACAAAUCACAUUGCCAGGGAUUUCCAACCGACC
((.(((.((((((((((((.((((.((((((..........)))))).)))).))))))).))))).))).))
```

hAgo3-top21   miR-378   3P

```
------------------------------------------ACUGGACUUGGAGUCAGAAGG---    16
------------------------------------------ACUGGACUUGGAGUCAGAAGGC--    270
-------------------------------------------CUGGACUUGGAGUCAGAAGGC--    27

AGGGCUCCUGACUCCAGGUCCUGUGUGUUACCUAGAAAUAGCACUGGACUUGGAGUCAGAAGGCCU
(((.((.((((((((((((((...((((((........)))))).)))))))))))))).)).)))
```

hAgo3-top22 miR-186 5P

```
--------------CAAAGAAUUCUCCUUUUGGGCUU-------------------------------------------------      109
--------------CAAAGAAUUCUCCUUUUGGGCU--------------------------------------------------      110
-------------CCAAAGAAUUCUCCUUUUGGGCU--------------------------------------------------      72

UGCUUGUAACUUUCCAAAGAAUUCUCCUUUUGGGCUUUCUGGUUUUAUUUUAAGCCCAAAGGUGAAUUUUUUGGGAAGUUUGAGCU
.(((((.(((((((((((((((((.(((((.((((((..............))))))))))).)))))))))))))))))))))).
```

hAgo3-top23 miR-29c 3P

```
-----------------------------------------------------UAGCACCAUUUGAAAUCGGUU--------------        23
-----------------------------------------------------UAGCACCAUcUGAAAUCGGUUA-------------        78
-----------------------------------------------------UAGCACCAUUUGAAAUCGGUUA-------------       243
-----------------------------------------------------UAGCACCAUUUGAAAUCGGUUAA------------        12

AUCUCUUACACAGGCUGACCGAUUUCUCCUGGUGUUCAGAGUCUGUUUUUGUCUAGCACCAUUUGAAAUCGGUUAUGAUGUAGGGGGA
.(((((((((((...(((((((((((...((((((((((((.....)))))...)))))))...))))))))))))).))))))))).
```

hAgo3-top24 miR-423-3P 3P

```
---------------------------------------------------AAGCUCGGUCUGAGGCCCCUCA---------------------   15
----------------------------------------------------AGCUCGGUCUGAGGCCCCUCAGU-------------------   33
----------------------------------------------------AGCUCGGUCUGAGGCCCCUCA---------------------   169
----------------------------------------------------AGCUCGGUCUGAGGCCCCUC----------------------   20
-----------------------------------------------------GCUCGGUCUGAGGCCCCUCA---------------------   10

AUAAAGGAAGUUAGGCUGAGGGGCAGAGAGCGAGACUUUUCUAUUUUCCAAAAGCUCGGUCUGAGGCCCCUCAGUCUUGCUUCCUAACCCGCGC
....(((((((.((((((((((((..(((.((((.(((((.........))))))))).)))...))))))))))))).))))))).........
```

hAgo3-top25 miR-130b* 5P

```
------------ACUCUUUCCCUGUUGCACUACU------------------------------------------------      252

GGCCUGCCCGACACUCUUUCCCUGUUGCACUACUAUAGGCCGCUGGGAAGCAGUGCAAUGAUGAAAGGGCAUCGGUCAGGUC
((((((.((((..(((((((..(((((((((.((.(((....)))...)).)))))))))..)))))))..)))).))))))
```

hAgo3-top26   miR-151-5p   5P

```
---------CUCGAGGAGCUCACAGUCUAGU-----------------------------------------------------------      84
----------UCGAGGAGCUCACAGUCUAGU-----------------------------------------------------------      72
----------UCGAGGAGCUCACAGUCUAGUA----------------------------------------------------------      78
---------CUCGAGGAGCUCACAGUCUAGUA----------------------------------------------------------      17

UUUCCUGCCCUCGAGGAGCUCACAGUCUAGUAUGUCUCAUCCCCUACUAGACUGAAGCUCCUUGAGGACAGGGAUGGUCAUACUCACCUC
.((((((.((((((((((((..((((((((((.(........).)))))))))).)))))))))))).)))))).(((.......)))..
```

hAgo3-top27 miR-501-3p 3P

```
--------------------------------------------------AAUGCACCCGGGCAAGGAUUCU------------   214
--------------------------------------------------AAUGCACCCGGGCAAGGAUUCUG-----------   14

GCUCUUCCUCUCUAAUCCUUUGUCCCUGGGUGAGAGUGCUUUCUGAAUGCAAUGCACCCGGGCAAGGAUUCUGAGAGGGUGAGC
((((.(((((((.(((((((.(.(((.(((((....(((.........)))...)))))))))))))))))..))))))).))))
```

hAgo3-top29 miR-221 3P

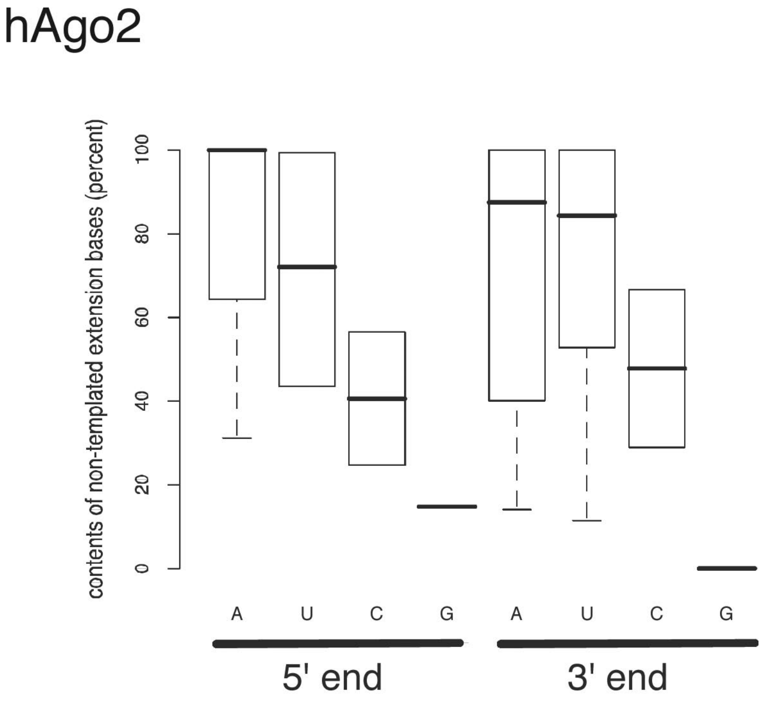
```
----------------------------------------------------------------AGCUACAUUGUCUGCUGGGUU-------------------------   10
----------------------------------------------------------------AGCUACAUUGUCUGCUGGGUUUC-----------------------   25
----------------------------------------------------------------AGCUACAUUGUCUGCUGGGUUU------------------------   131

UGAACAUCCAGGUCUGGGGCAUGAACCUGGCAUACAAUGUAGAUUUCUGUGUUCGUUAGGCAACAGCUACAUUGUCUGCUGGGUUUCAGGCUACCUGGAAACAUGUUCUC
.((((((((((((....(((.((((((..(((.(((((((((....(((((((.....))).))))))))))))).)))..)).)))).))))))))))....)))))..
```

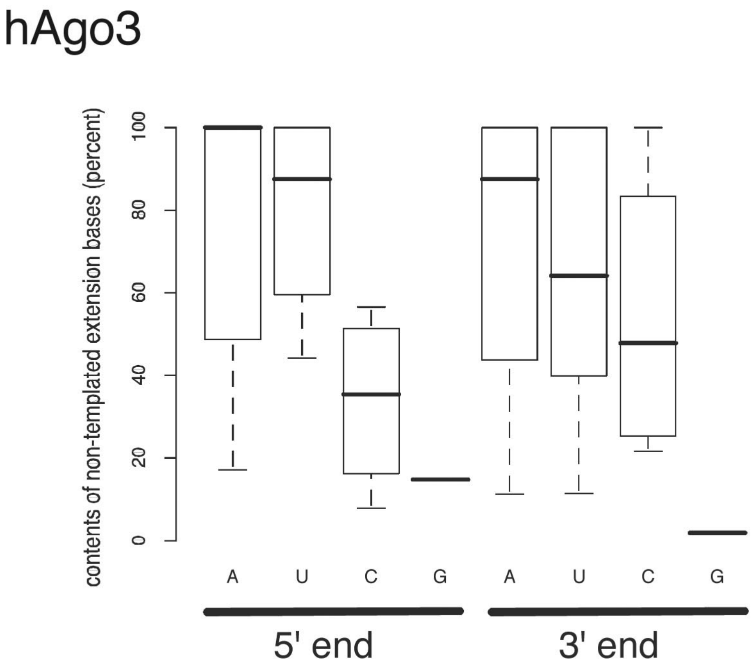
hAgo3-top21   miR-378   3P

```
------------------------------------------ACUGGACUUGGAGUCAGAAGG---     16
------------------------------------------ACUGGACUUGGAGUCAGAAGGC--     270
-------------------------------------------CUGGACUUGGAGUCAGAAGGC--     27

AGGGCUCCUGACUCCAGGUCCUGUGUGUUACCUAGAAAUAGCACUGGACUUGGAGUCAGAAGGCCU
(((.((.((((((((((((((...((((((........)))))).))))))))))))))).)).)))
```



hAgo2



hAgo3

Fig. S6. Distributions of nontemplated bases found in the top 30 miRNAs in the hAgo2 and hAgo3 libraries. Only variants that appeared a total of 10 times or more were considered as subjects for this statistical study. Apparent preference to adenine and uracil is shown, while nontemplated guanine is extremely rare.

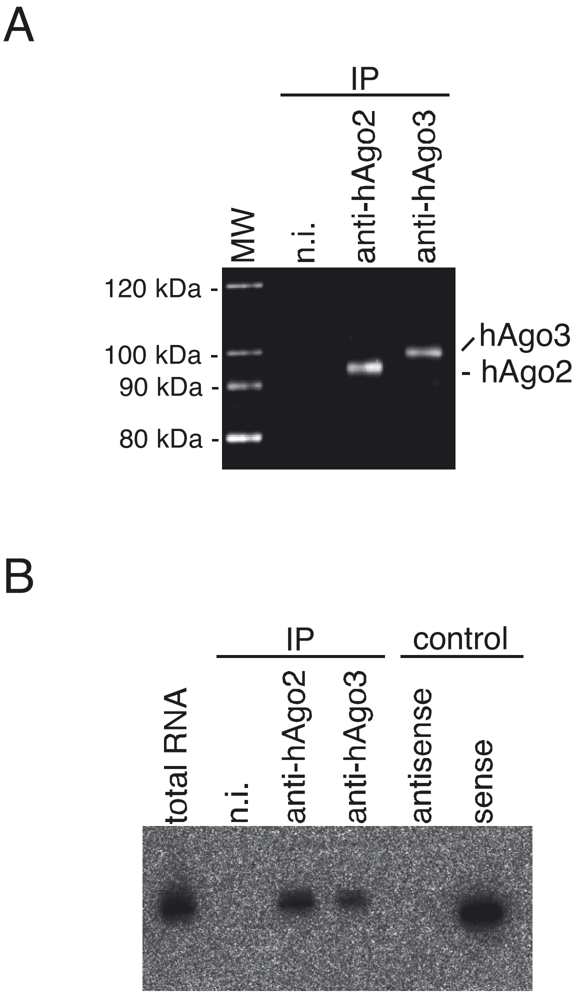


Fig. S7. (*A*) Immunoprecipitates from Jurkat cells using anti-hAgo2 and -hAgo3 stained with SYPRO Ruby (Molecular Probes). hAgo3 was immunopurified from three volumes of the Jurkat cell lysate that was used for hAgo2 immunopurification, where the amount of hAgo3 immunopurified was ≈80% of that of hAgo2. (*B*) RNAs purified from the immunoprecipitates prepared as in *A* confirmed with a DNA oligo recognizing miR-150. Total RNAs from Jurkat cells, and DNA oligos with sequences of miR-150 (sense) and miR-150* (antisense) were used as controls.

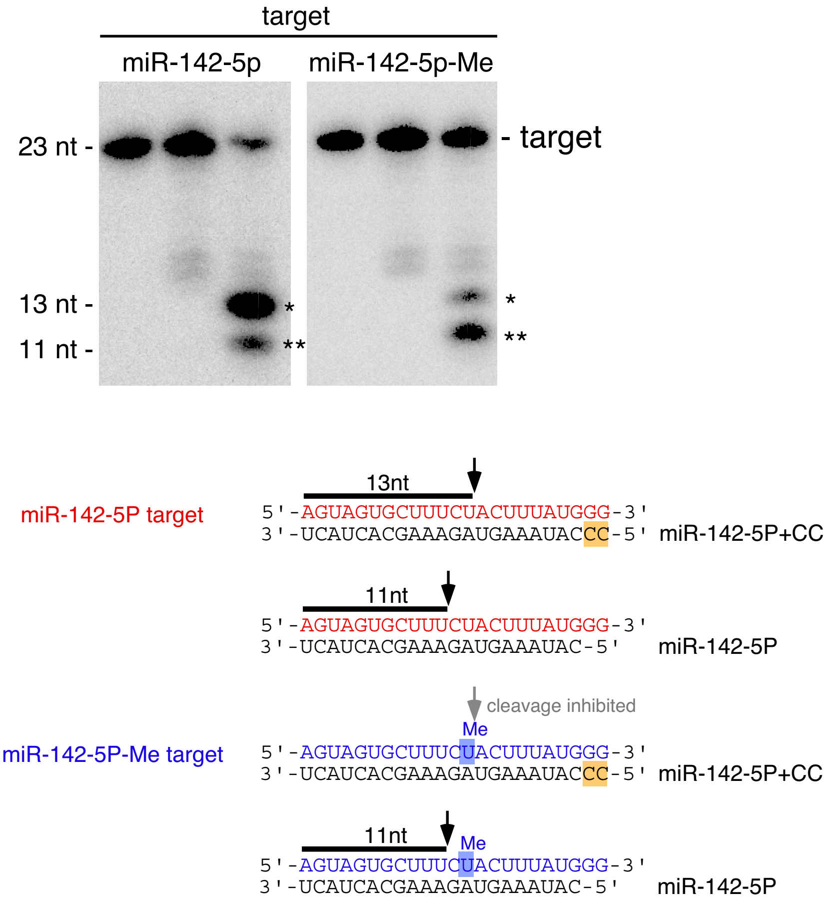


Fig. S8. *In vitro* target RNA cleavage assays using miR-142-5p target. (*Upper Left*) Target RNA harboring a sequence completely complementary to miR-142-5p (miR-142-5p target; 23 nt; the sequence is shown below) performed by using hAgo2 immunoprecipitated complexes from Jurkat cells. The hAgo2 complex predominantly cleaved the target at two major sites; one corresponding to the site of the cleavage directed by registered miR-142-5p (double asterisks; 11 nt), and the other corresponding to the site of the cleavage directed by one very abundant miR-142-5p variant that has two additional nucleotides (mostly CC) at the 5′ end (miR-142-5p+CC) (asterisk; 13 nt). (*Upper Right*) miR-142-5p target modified with a methyl group at the expected cleavage site by miR-142-5p+CC [miR-142-5p-Me target; nucleotide 13 (U, highlighted in blue) was methylated; see *Lower*] showed strong resistance to miR-142-5p+CC but not to registered miR-142-5 cleavage.

Table S1. Top 30 miRNAs appearing in the hAgo2 and hAgo3 libraries

miRNA	pre-miRNA arm	Most abundant sequence (mas)	Count of mas/total count	miRBase sequence = mas (Y, yes; N, no)
hAgo2				
miR-150	5p	UCUCCCAACCCUUGUACCAGUG	4,478/6,713	Y
miR-99b	5p	CACCCGUAGAACCGACCUGCG	4,330/5,285	Y
miR-142-3p	3p	GUAGUGUUUCCUACUUUAUGGA	2,952/4,359	N
miR-21	5p	UAGCUUAUCAGACUGAUGUUGA	2,053/4,237	Y
miR-92a	3p	UAUUGCACUUGUCCCGGCCUGU	1,855/2,918	Y
miR-342-3p	3p	UCUCACACAGAAAUCGCACCCGU	1,273/2,533	Y
miR-484	5p	UCAGGCUCAGUCCCCUCCCGAU	1,573/2,401	Y
miR-142-5p	5p	CCCAUAAAGUAGAAAGCACUAC	1,831/2,220	N
miR-425	5p	AAUGACACGAUCAGUCCCGUUGA	714/1,563	Y
miR-532-5p	5p	CAUGCCUUGAGUGUAGGACCGU	1,036/1,372	Y
miR-17*	3p	ACUGCAGUGAAGGCACUUGUAGC	423/1,077	N
miR-210	3p	CUGUGCGUGUGACAGCGGCUGA	721/935	Y
miR-16	5p	UAGCAGCACGUAAAUAUUGGCG	641/779	Y
miR-25	3p	CAUUGCACUUGUCUCGGUCUGA	605/731	Y
miR-423-3p	3p	AGCUCGGUCUGAGGCCCCUCAGU	439/698	Y
miR-92b	3p	UAUUGCACUCGUCCCGGCCUCC	314/682	Y
let-7d*	3p	CUAUACGACCUGCUGCCUUUCU	396/667	Y
miR-191	5p	CAACGGAAUCCCAAAAGCAGCU	264/644	N
miR-128-1/-2	3p	UCACAGUGAACCGGUCUCUUU	442/495	Y
miR-28-3p	3p	CACUAGAUUGUGAGCUCCUGGA	311/484	Y
miR-221	3p	AGCUACAUUGUCUGCUGGGUUU	254/444	N
miR-192	5p	CUGACCUAUGAAUUGACAGCC	110/418	Y
miR-15a	5p	UAGCAGCACAUAAUGGUUUGUG	233/390	Y
miR-941	3p	CACCCGGCUGUGUGCACAUGUGC	309/386	Y
miR-197	3p	UUCACCACCUUCUCCACCCAGC	243/381	Y
miR-29c	3p	UAGCACCAUUUGAAAUCGGUUA	301/363	Y
miR-766	3p	ACUCCAGCCCCACAGCCUCAGC	173/348	Y
miR-345	5p	GCUGACUCCUAGUCCAGGGCUC	134/327	Y
miR-93*	3p	ACUGCUGAGCUAGCACUUCCCGA	122/302	N
miR-501-3p	3p	AAUGCACCCGGGCAAGGAUUCU	199/299	Y
hAgo3				
miR-142-3p	3p	GUAGUGUUUCCUACUUUAUGG	12,636/18,409	Y
miR-150	5p	UCUCCCAACCCUUGUACCAGUG	9,951/12,732	Y
miR-92b	3p	UAUUGCACUCGUCCCGGCCUCC	4,835/8,324	Y
miR-425	5p	AAUGACACGAUCAGUCCCGUUGA	4,070/5,022	Y
miR-532-5p	5p	CAUGCCUUGAGUGUAGGACCGU	3,653/4,753	Y
miR-92a	3p	UAUUGCACUUGUCCCGGCCUGU	2,171/3,131	Y
miR-629*	3p	GUUCUCCCAACGUAAGCCCAGC	1,318/3,122	Y
miR-210	3p	CUGUGCGUGUGACAGCGGCUGA	2,561/2,893	Y
miR-484	5p	UCAGGCUCAGUCCCCUCCCGAU	813/2,207	Y
miR-191	5p	CAACGGAAUCCCAAAAGCAGCU	1,273/1,847	N
miR-21	5p	UAGCUUAUCAGACUGAUGUUGA	1,191/1,412	Y
miR-99b	5p	CACCCGUAGAACCGACCUGCG	717/830	Y
miR-93*	3p	ACUGCUGAGCUAGCACUUCCCGA	367/767	N
miR-222	3p	AGCUACAUCUGGCUACUGGGUC	332/720	N
let-7d*	3p	CUAUACGACCUGCUGCCUUUCU	539/656	Y
miR-142-5p	5p	CAUAAAGUAGAAAGCACUACTA	227/619	N
miR-25	3p	CAUUGCACUUGUCUCGGUCUGA	429/477	Y
miR-26a	5p	UUCAAGUAAUCCAGGAUAGGCU	222/432	Y
miR-324-5p	5p	CGCAUCCCCUAGGGCAUUGGU	360/426	N
miR-23a	3p	AUCACAUUGCCAGGGAUUUCCA	265/388	N
miR-378	3p	ACUGGACUUGGAGUCAGAAGGC	270/364	N
miR-186	5p	CAAAGAAUUCUCCUUUUGGGCU	110/320	N
miR-29c	3p	UAGCACCAUUUGAAAUCGGUUA	243/302	Y
miR-423-3p	3p	AGCUCGGUCUGAGGCCCCUCA	169/294	N
miR-130b*	5p	ACUCUUUCCCUGUUGCACUACU	252/291	N
miR-151-5p	5p	CUCGAGGAGCUCACAGUCUAGU	84/288	N
miR-501-3p	3p	AAUGCACCCGGGCAAGGAUUCU	214/265	Y
let-7d	5p	AGAGGUAGUAGGUUGCAUAGUU	166/227	Y
miR-221	3p	AGCUACAUUGUCUGCUGGGUUU	131/194	N
miR-28-3p	3p	CACUAGAUUGUGAGCUCCUGGA	151/193	Y

Shown are all kinds of miRNAs identified in the hAgo2 and hAgo3 small RNA libraries.

Other Supporting Information Files

Dataset S1

Dataset S2