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Argonaute-3 activates the let-7a passenger strand microRNA

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Supplementary Figure Legends 1 - 15

Supplementary Figures 1 - 15

Supplementary Tables 1 - 2

Supplementary Figure Legends

Suppl. Figure 1 Argonaute-3 overexpression increases ectopic let-7a-3p expression. HeLa cells were cotransfected with a construct encoding let-7a-3 pri-miRNA and Ago1-4. Mature miRNA expression was determined by qRT-PCR analysis for let-7a-5p (white bars) and let-7a-3p (black bars) and the let-7a-5p / -3p ratio (grey bars) was calculated. Depicted is the mean expression (+SEM) of three independent experiments as compared to the EGFP-transfected control cells.

Suppl. Figure 2 Argonaute-3 effect is restricted to let-7a-3p. Mature endogenous expression for several miRNAs in HEK293 cells transfected with Ago1-4 proteins was determined by qRT-PCR analysis. Depicted is the mean expression (+SEM) of three independent experiments as compared to the EGFP-transfected control cells.

Suppl. Figure 3 Argonaute-3 effect is restricted to let-7a-3p. Mature endogenous expression for several miRNA guide and passenger strand pairs in HEK293 cells transfected with Ago1-4 proteins was determined by qRT-PCR analysis. Depicted is the mean expression (+SEM) of three independent experiments as compared to the EGFP-transfected control cells.

Suppl. Figure 4 Argonaute-3 effect is restricted to let-7a-3p. HEK293 cells were transfected with two different siRNAs against Argonaute-3 expression and expression of several miRNA guide and passenger strand pairs was determined by qRT-PCR analysis. Depicted is the mean expression (+SEM) of three independent experiments as compared to the control-siRNA-transfected cells.

Suppl. Figure 5 Let-7a-3p might be an active miRNA targeting RAB10. (A) HEK293 cells were cotransfected with let-7a, Ago3 and a reporter construct encoding luciferase fused to the 3´-UTR of RAB10 as indicated (black bars). Constructs with two mismatches in the seed binding site served as negative controls (grey bars). Renilla luciferase was cotransfected for normalization. Depicted is the mean fold repression (+SEM) of the respective endogenous targets compared to control (control miRNA / EGFP transfections). **(B)** HEK293 cells were cotransfected with let-7a and Ago3 for 72 hours and RAB10 protein expression levels were detected by Western Blotting. Tubulin served as loading control. **(C)** Quantification of Western Blots performed in (B). Shown is the mean +SEM of three independent experiments.

Suppl. Figure 6 Overexpression of Argonaute-3 does not specifically increase miRNA precursor levels. Ectopic **(A, C)** and endogenous **(B, D)** miRNA precursor expression in HEK293 cells transfected with Argonaute proteins was determined. Pri-let-7a expression was measured by qRT-PCR **(A, B)**, pre-let-7a and mature let-7a-3p expression levels were detected via Northern Blotting **(C, D)**. Reprobing of the blot for U6 snRNA served as loading control. **(E)** HEK293 cells were cotransfected with a let-7a miRNA duplex and Ago1 and Ago3. Mature miRNA expression was determined by qRT-PCR analysis for let-7a-5p (white bars) and let-7a-3p (black bars) and the let-7a-5p / -3p ratio (grey bars) was calculated. Depicted is the mean expression (+SEM) of three independent experiments as compared to the respective EGFP-transfected control cells.

Suppl. Figure 7 Argonaute-3 effect is independent of the terminal loop structure. (A) The terminal loop structure of let-7a and miR-193a was swapped and let-7a+loop miR-193 and miR-193a+loop let-7a mutants cloned. **(B-E)** Mature miRNA expression in HEK293 cells cotransfected with constructs encoding let-7a-3 WT **(B)**, miR-193a WT **(C)**, let-7a+loop-miR-193a **(D)** and miR-193a+loop-let-7a **(E)** and Argonaute proteins 1-4 was

determined by qRT-PCR analysis. 5p (white bars) and 3p (black bars) expression was determined for let-7a and miR-193a, respectively, and 5p / 3p ratios (grey bars) were calculated. Depicted is the mean expression (+SEM) of three independent experiments as compared to the EGFP-transfected cells.

Suppl. Figure 8 Loss of Ago2 decreases only let-7a-5p expression. Mature endogenous let-7a-5p (white bars) and let-7a-3p (black bars) expression was determined by qRT-PCR analysis in mouse embryonic fibroblasts lacking the expression of Argonaute-2 and let-7a-5p / -3p ratios were calculated (grey bars). Depicted is the mean expression (+SEM) of three independent experiments as compared to wildtype cells.

Suppl. Figure 9 Thermodynamic stability of the terminal basepair influences the expression of the guide strand and the passenger strand. HEK293 cells were cotransfected with Ago1-4 and constructs encoding the let-7c **(A)** and miR-27b **(B)** pri-miRNAs. The wildtype pri-miRNAs (white bars) as well as mutants of the primary basepair of the 5'-arm affecting thermodynamic stability (black bars) were compared (MM = mismatch; PM = perfect match). Mature guide and passenger strand expression was determined by qRT-PCR analysis. Depicted is the mean expression (+SEM) of three independent experiments normalized to the EGFP-transfected control cells.

Suppl. Figure 10 Mismatched base-pairing in the center of the miRNA duplex does not abrogate the Ago3 effect. HEK293 cells were cotransfected with Ago1-4 and constructs encoding the pri-let-7a that were either wildtype or mutated in the central region of the miRNA-duplex. Mature guide and passenger strand expression of let-7-3p **(A)** and let-7a-5p **(B)** was determined by qRT-PCR analysis. Depicted is the mean expression (+SEM) of three independent experiments normalized to the EGFP-transfected control cells.

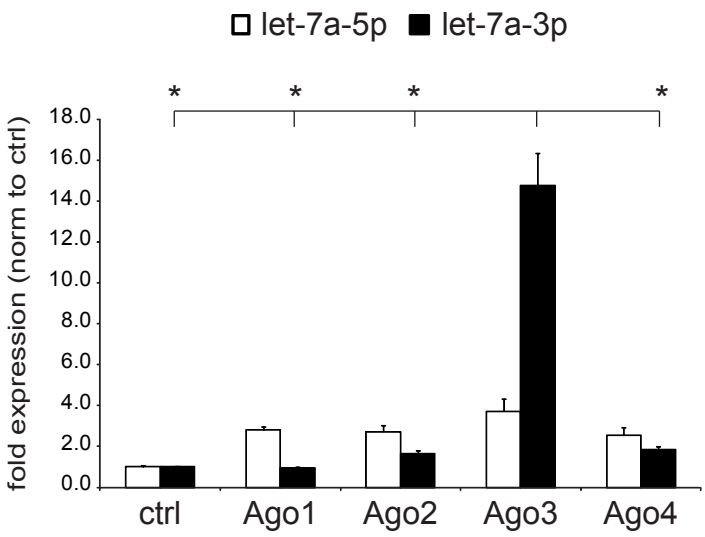
Suppl. Figure 11 Argonaute protein expression. Expression of FLAG/HA-tagged Argonaute proteins in HEK293 cells was analyzed by Western Blot using α-HA and α-Tubulin (loading control) antibodies.

Suppl. Figure 12 Argonaute domain swap mutants do not have a specific effect on miR-20a guide and passenger strand expression. Argonaute domain swap mutants were cloned to exchange individual domains of Argonaute-1 and Argonaute-3. These mutants were cotransfected together with a construct encoding the miR-20a pri-miRNA. miR-20a-5p (white bars) and -3p (black bars) expression was detected by qRT-PCR. Depicted is the mean expression (+SEM) of three independent experiments normalized to the EGFP-transfected control cells.

Suppl. Figure 13 Argonaute-3 displays increased let-7a-3p / -5p binding affinity. **(A)** Schematic overview of the coimmunoprecipitation experiments used to detect association of miRNAs with Argonaute proteins. **(B, C)** HEK293 cells were cotransfected with FLAG-tagged Argonaute proteins and a construct encoding let-7a-3 pri-miRNA for 48 hours before cells were lysed and Argonaute proteins were coupled to FLAG-Agarose beads that had been pre-blocked with BSA and tRNA. Ectopic **(B)** and endogenous **(C)** let-7a-3p / -5p binding affinity (IP versus Input) to the appropriate Argonaute proteins was determined by qRT-PCR analysis. Depicted is the mean expression (+SEM) of three independent experiments compared to Ago3. **(D)** HEK293 cells were cotransfected with constructs encoding FLAG-HA-tagged Ago1, Ago3 and Ago3deltaPAZ. Mature miRNA expression was determined by qRT-PCR analysis for let-7a-5p (white bars) and let-7a-3p (black bars). **(E)** Expression of the Ago constructs was validated by Western Blotting.

Suppl. Figure 14 Endogenous let-7a-3p expression and regulation. (A) MiRNA expression of let-7a-3 and other miRNA guide and passenger strands and mirtrons was analyzed in HEK293 cells by qRT-PCR analysis. Shown are Ct-values with lower values indicating exponentially higher expression. **(B)** K562 cells were treated with Hemin for 96 hours and let-7a-5p (white bars), let-7a-3p (black bars) and Argonaute-3 (grey bars) expression was determined by qRT-PCR analysis. Depicted is the mean expression (+SEM) of three independent experiments as compared to untreated K562 cells.

Suppl. Figure 15 5' terminal nucleotide identity of mature miRNAs tested for Ago3 effect throughout this manuscript.


let-7a-5p
let-7a-3p
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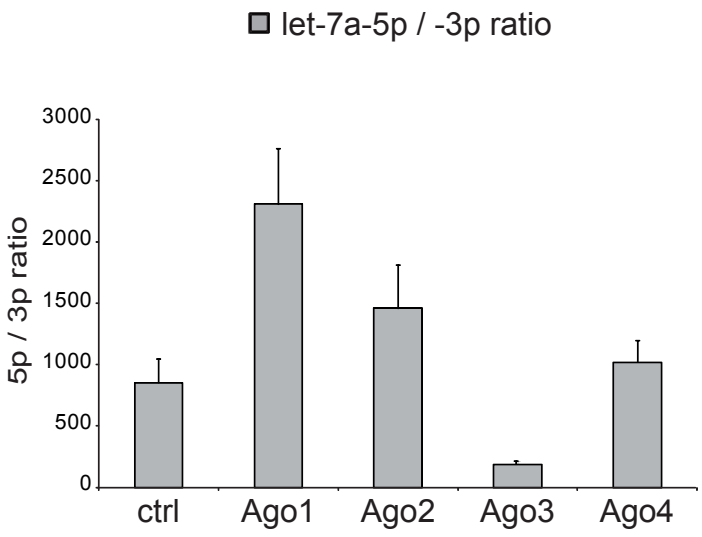
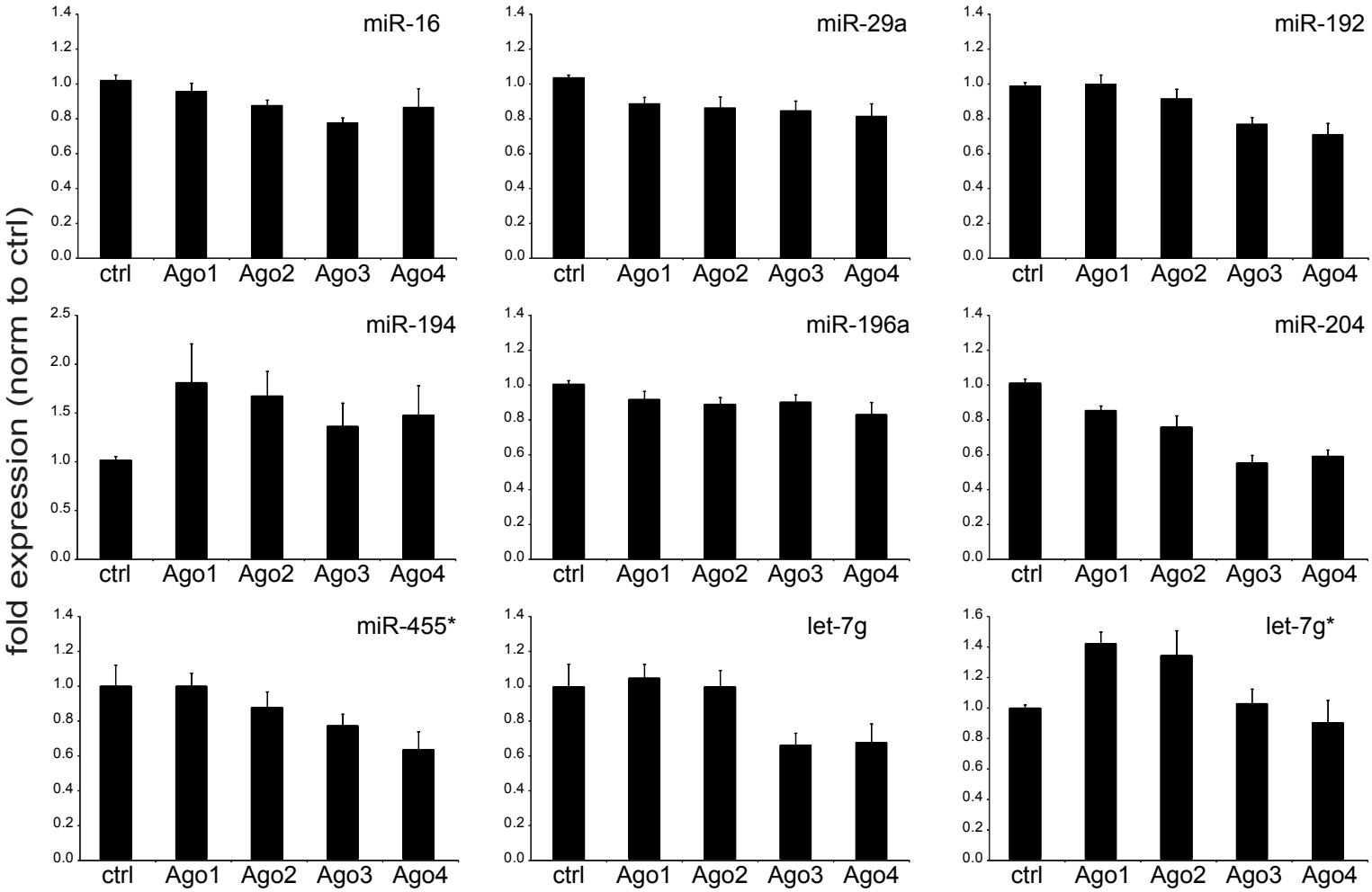
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
*
*
*
*
ctrl
Ago1
Ago2
Ago3
Ago4


let-7a-5p / -3p ratio
5p / 3p ratio
3000
2500
2000
1500
1000
500
0
ctrl
Ago1
Ago2
Ago3
Ago4

Suppl Fig 1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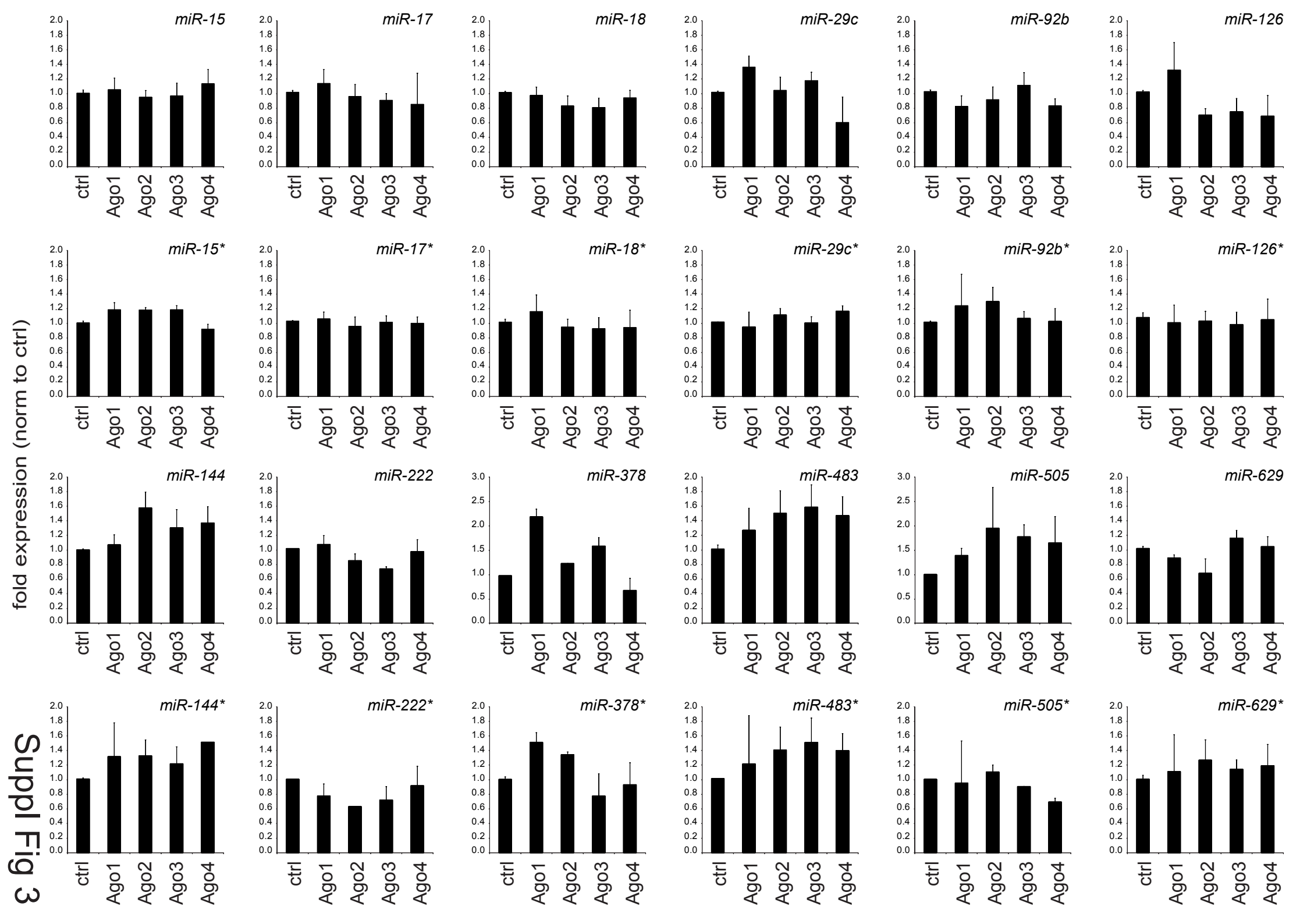
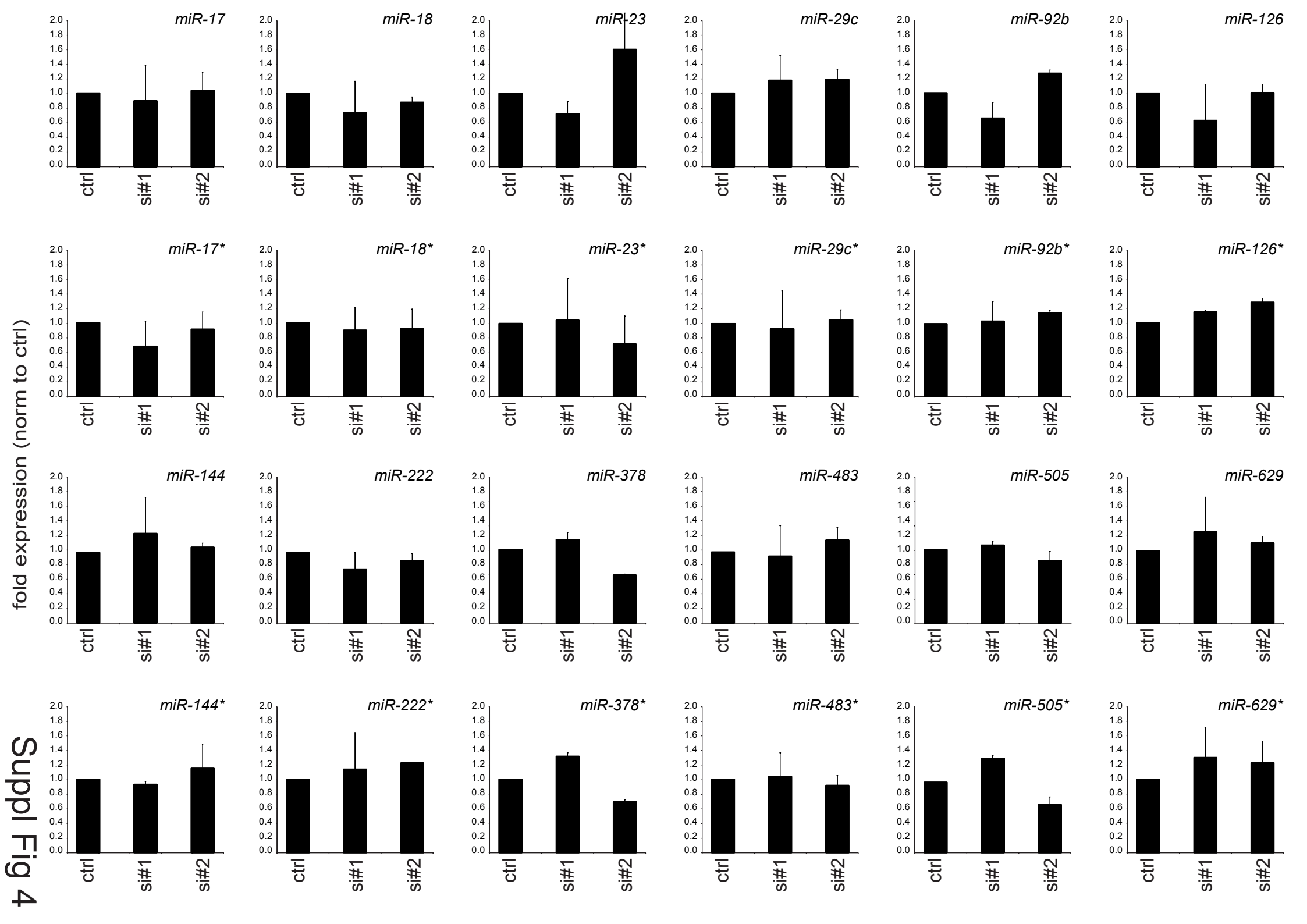
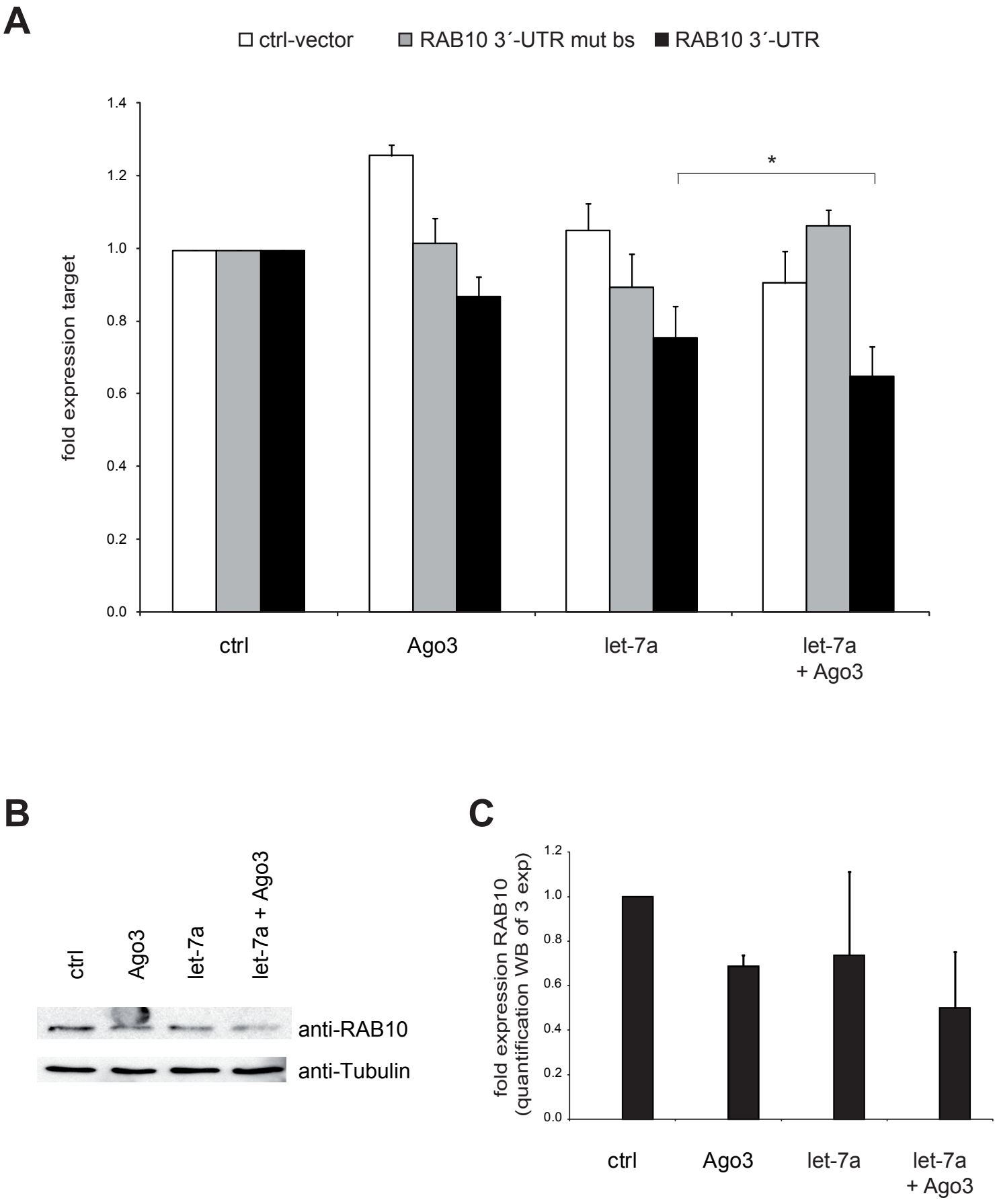
miR-29a
miR-192
miR-194
miR-196a
miR-204
miR-455*
let-7g
let-7g*
ctrl
Ago1
Ago2
Ago3
Ago4

Suppl Fig 2

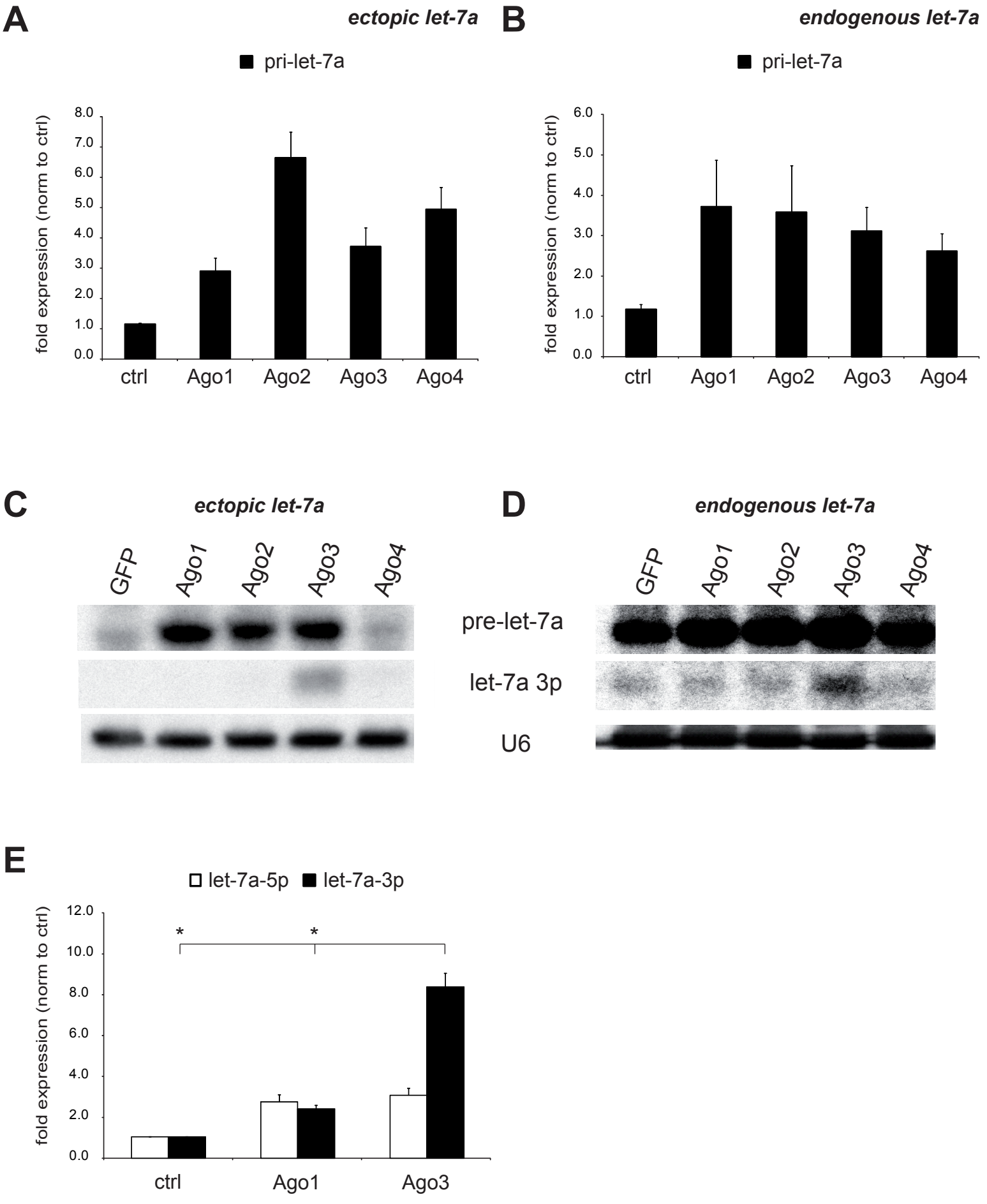

Suppl Fig 3
fold expression (norm to ctrl)
miR-15
miR-17
miR-18
miR-29c
miR-92b
miR-126
miR-15*
miR-17*
miR-18*
miR-29c*
miR-92b*
miR-126*
miR-144
miR-222
miR-378
miR-483
miR-505
miR-629
miR-144*
miR-222*
miR-378*
miR-483*
miR-505*
miR-629*
ctrl
Ago1
Ago2
Ago3
Ago4


miR-17
miR-18
miR-23
miR-29c
miR-92b
miR-126
miR-17*
miR-18*
miR-23*
miR-29c*
miR-92b*
miR-126*
miR-144
miR-222
miR-378
miR-483
miR-505
miR-629
miR-144*
miR-222*
miR-378*
miR-483*
miR-505*
miR-629*
fold expression (norm to ctrl)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ctrl
si#1
si#2

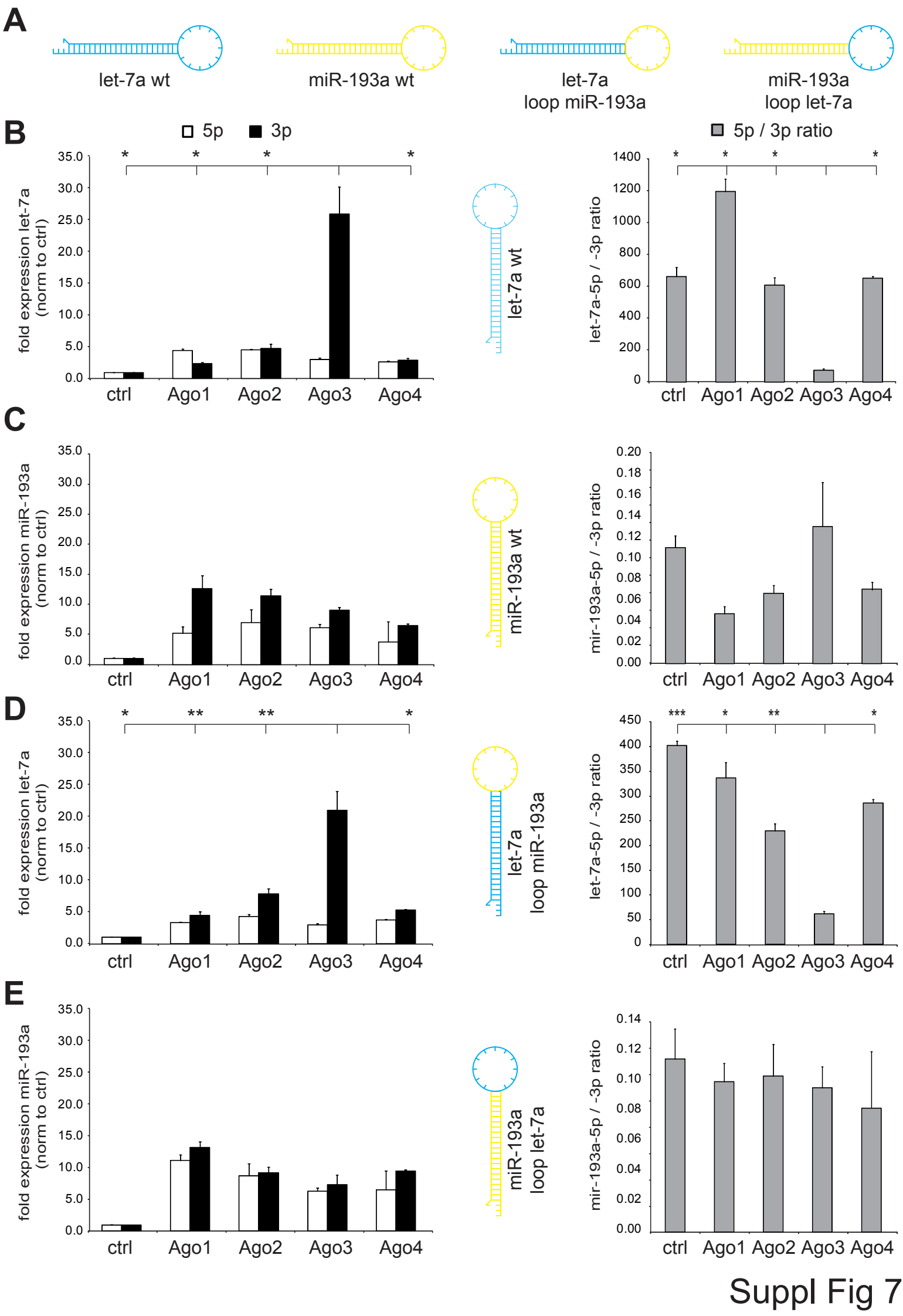
Suppl Fig 4

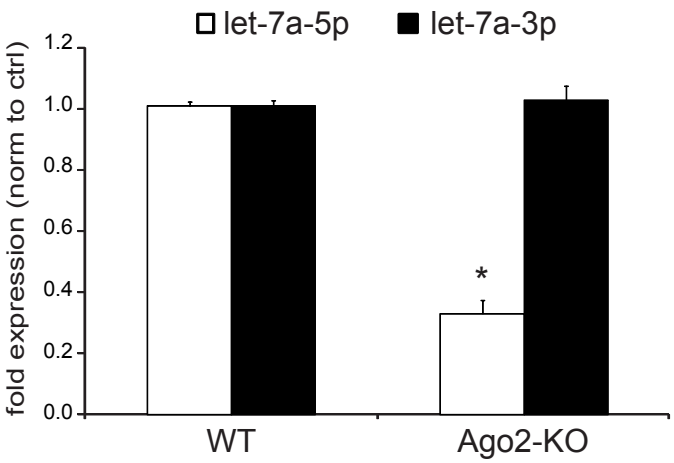


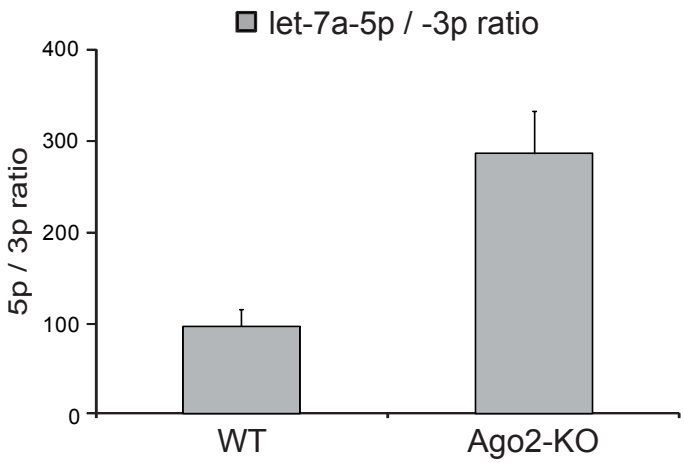
Suppl. Figure 5


A
ectopic let-7a
pri-let-7a
fold expression (norm to ctrl)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ctrl
Ago1
Ago2
Ago3
Ago4
B
endogenous let-7a
pri-let-7a
fold expression (norm to ctrl)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ctrl
Ago1
Ago2
Ago3
Ago4
C
ectopic let-7a
GFP
Ago1
Ago2
Ago3
Ago4
D
endogenous let-7a
GFP
Ago1
Ago2
Ago3
Ago4
pre-let-7a
let-7a 3p
U6
E
let-7a-5p
let-7a-3p
fold expression (norm to ctrl)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
*
*
ctrl
Ago1
Ago3

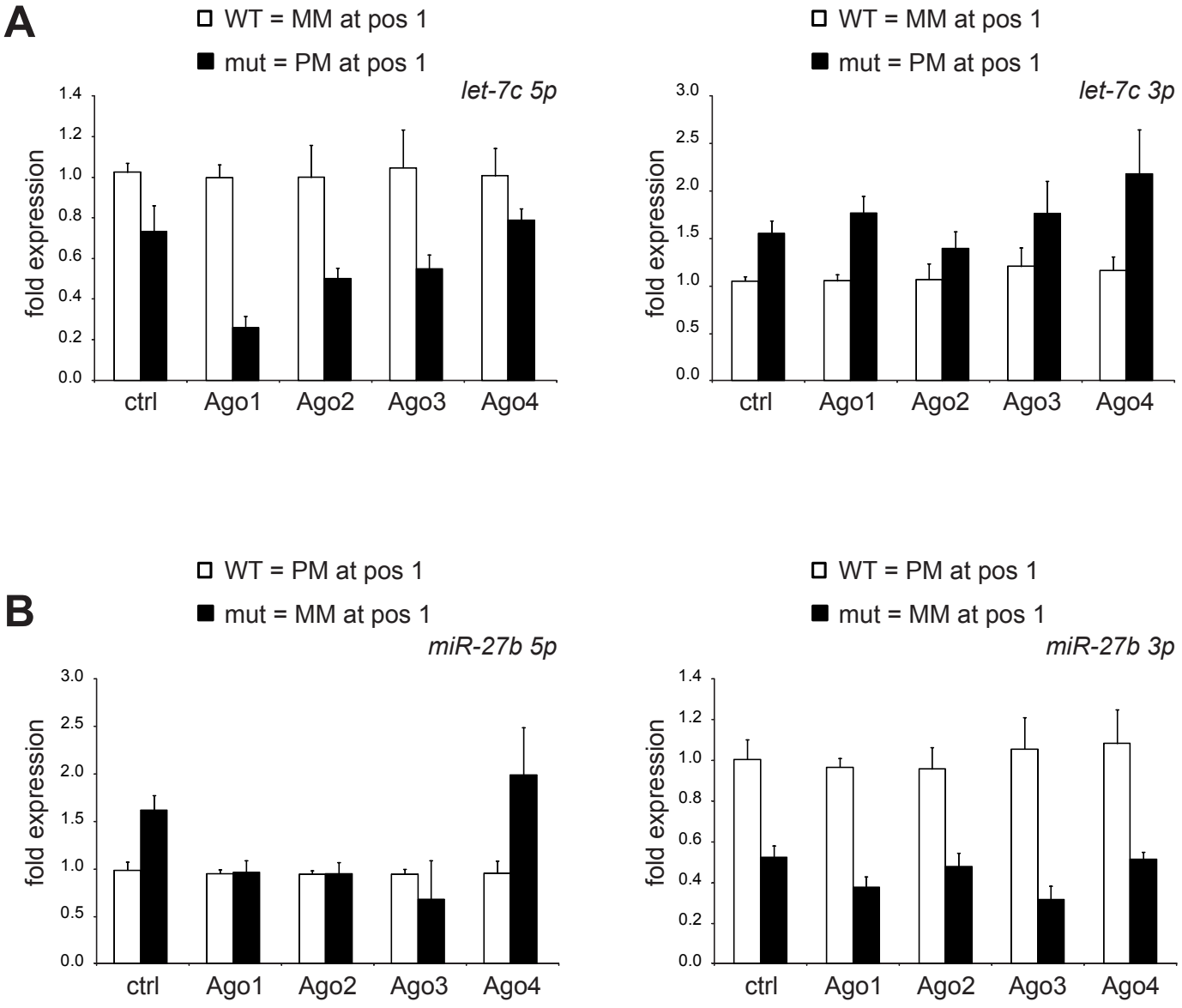
Suppl Fig 6


A
let-7a wt
miR-193a wt
let-7a loop miR-193a
miR-193a loop let-7a
B
5p
3p
5p / 3p ratio
fold expression let-7a (norm to ctrl)
let-7a wt
let-7a-5p / -3p ratio
ctrl
Ago1
Ago2
Ago3
Ago4
C
fold expression miR-193a (norm to ctrl)
miR-193a wt
mir-193a-5p / -3p ratio
D
let-7a loop miR-193a
E
miR-193a loop let-7a
Suppl Fig 7

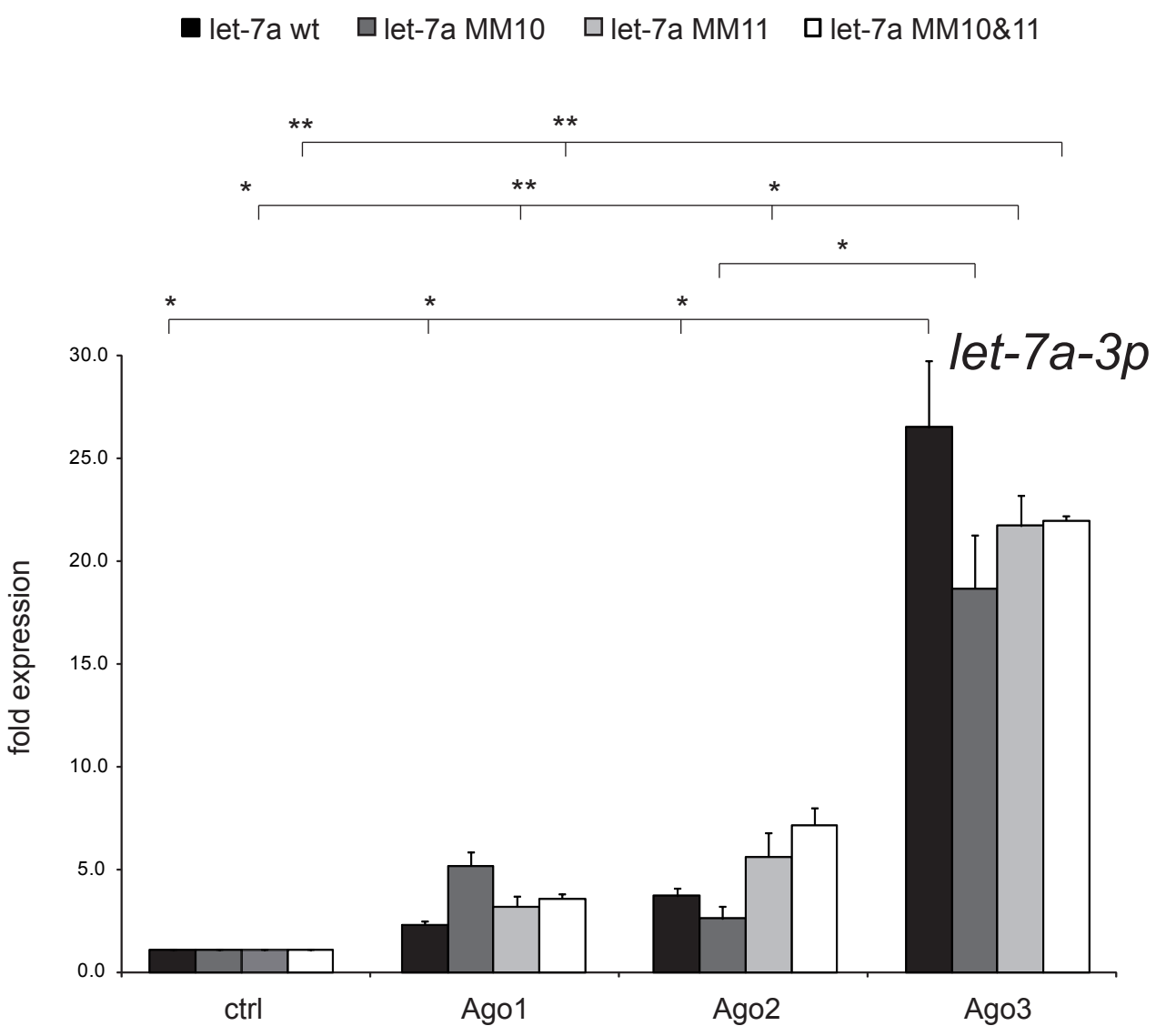

let-7a-5p
let-7a-3p
fold expression (norm to ctrl)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
WT
Ago2-KO

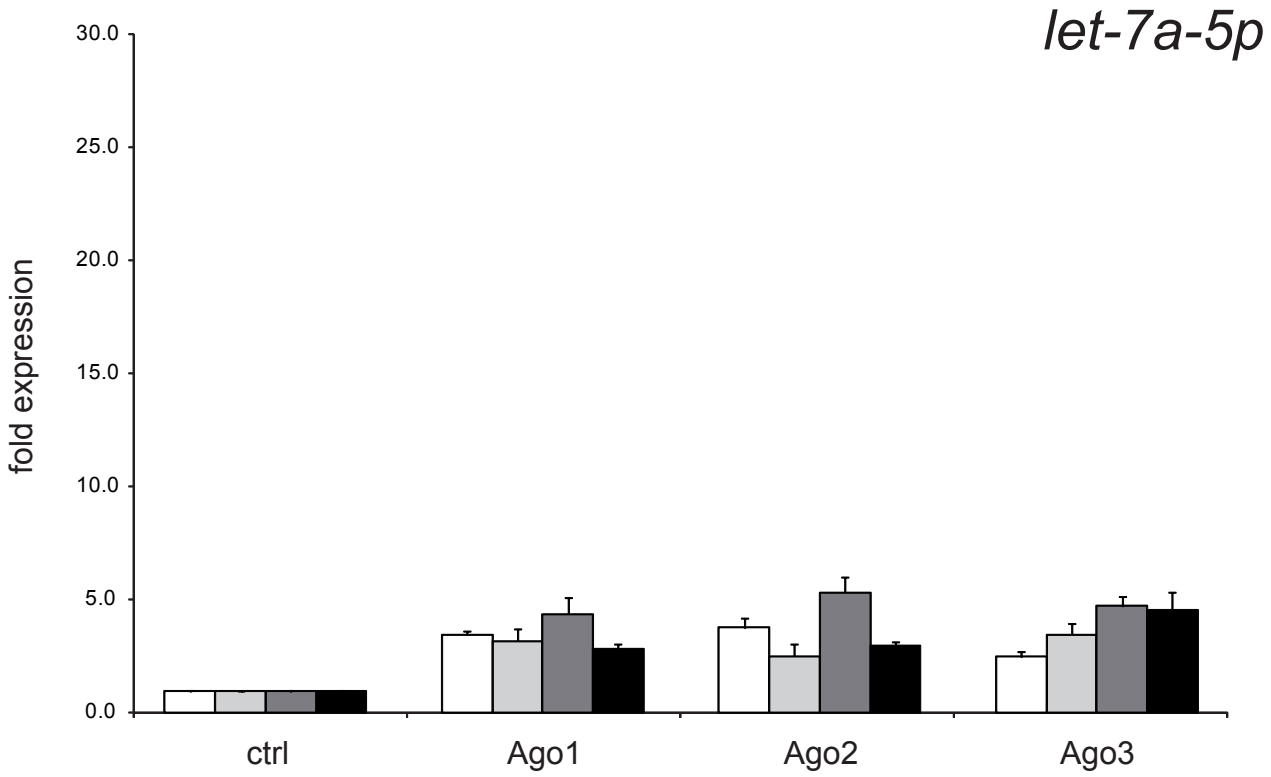

let-7a-5p / -3p ratio
5p / 3p ratio
400
300
200
100
0
WT
Ago2-KO

Suppl Fig 8

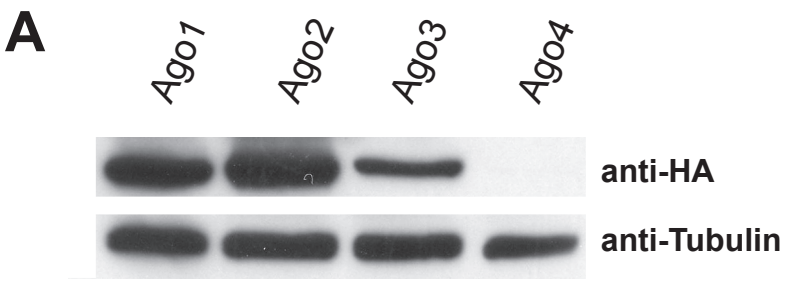

A
WT = MM at pos 1
mut = PM at pos 1
let-7c 5p
fold expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ctrl
Ago1
Ago2
Ago3
Ago4
WT = MM at pos 1
mut = PM at pos 1
let-7c 3p
fold expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ctrl
Ago1
Ago2
Ago3
Ago4
B
WT = PM at pos 1
mut = MM at pos 1
miR-27b 5p
fold expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ctrl
Ago1
Ago2
Ago3
Ago4
WT = PM at pos 1
mut = MM at pos 1
miR-27b 3p
fold expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ctrl
Ago1
Ago2
Ago3
Ago4

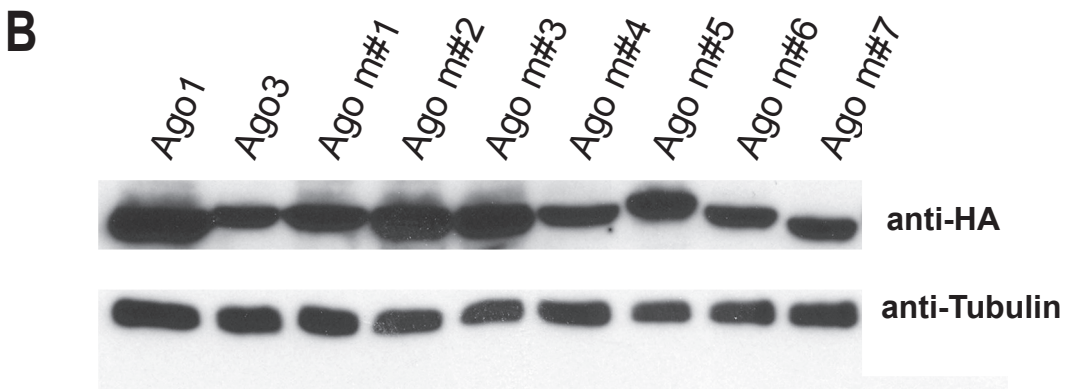
Suppl Fig 9


let-7a wt
let-7a MM10
let-7a MM11
let-7a MM10&11
**
**
*
**
*
*
*
*
let-7a-3p
fold expression
30.0
25.0
20.0
15.0
10.0
5.0
0.0
ctrl
Ago1
Ago2
Ago3

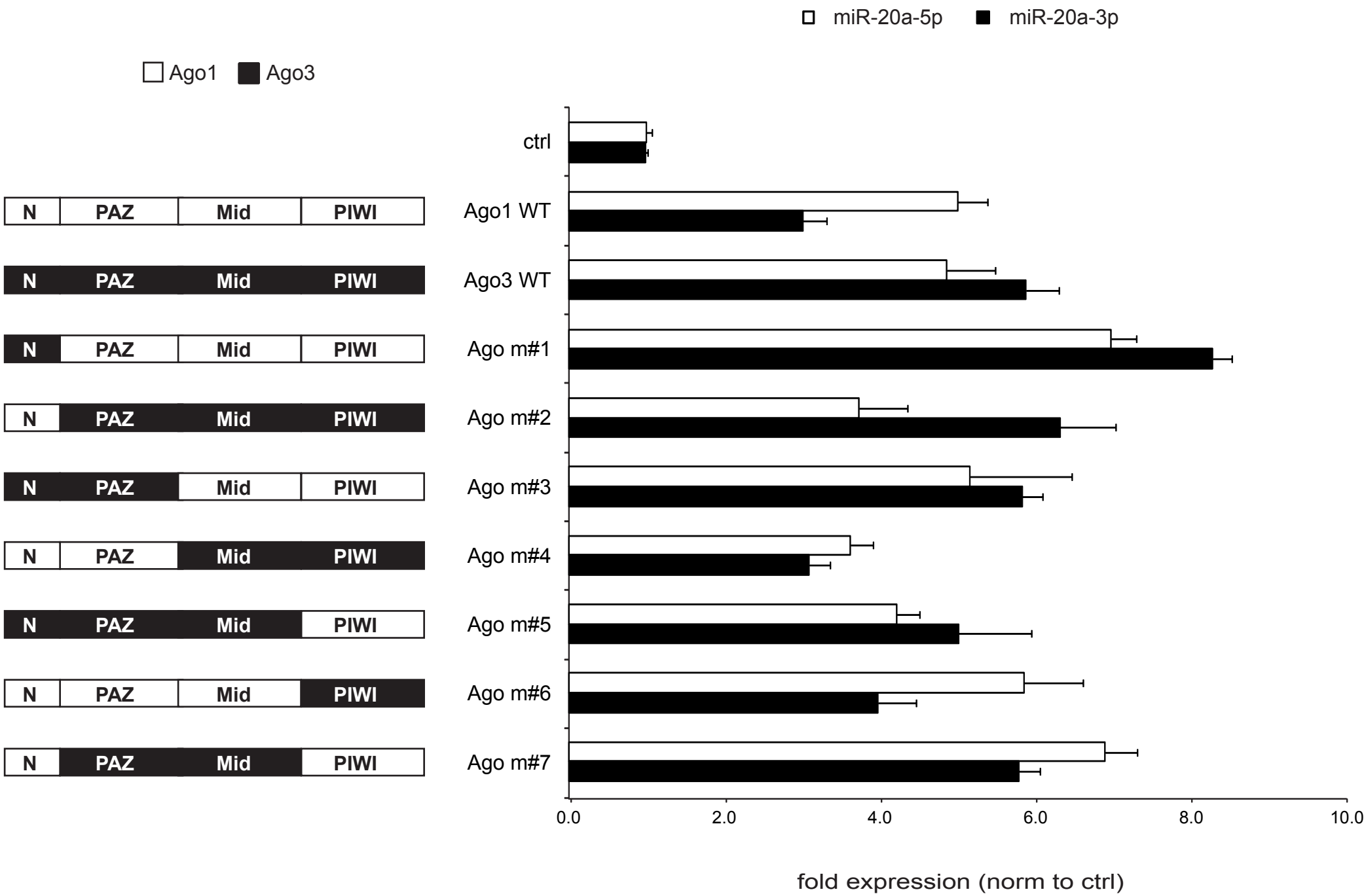

let-7a-5p
fold expression
30.0
25.0
20.0
15.0
10.0
5.0
0.0
ctrl
Ago1
Ago2
Ago3

Suppl Fig 10

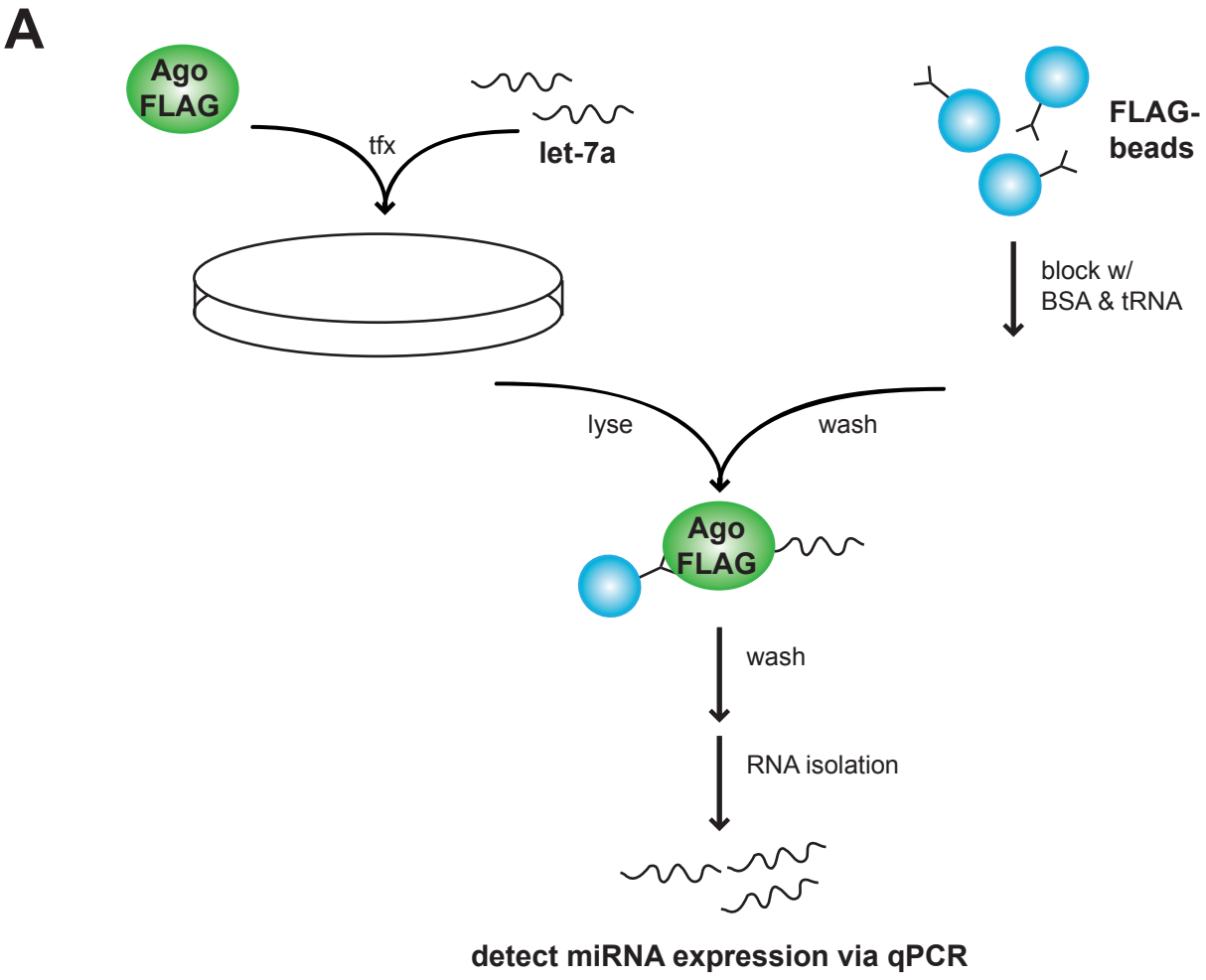

A
Ago1
Ago2
Ago3
Ago4
anti-HA
anti-Tubulin

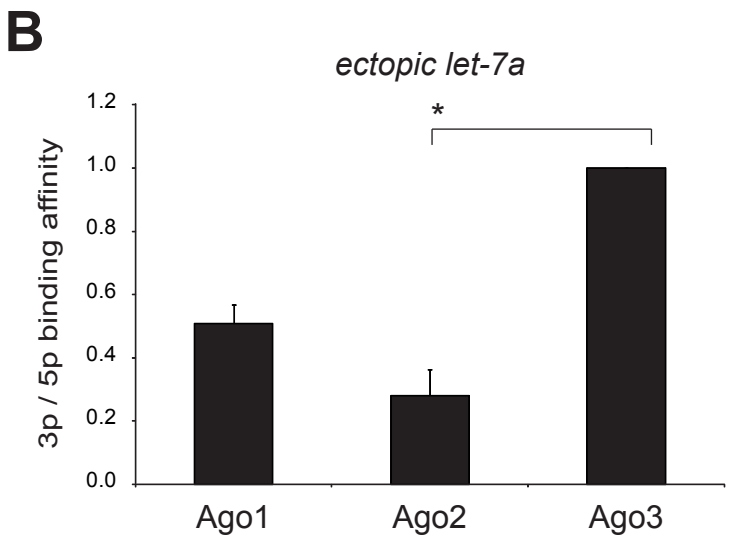

B
Ago1
Ago3
Ago m#1
Ago m#2
Ago m#3
Ago m#4
Ago m#5
Ago m#6
Ago m#7
anti-HA
anti-Tubulin

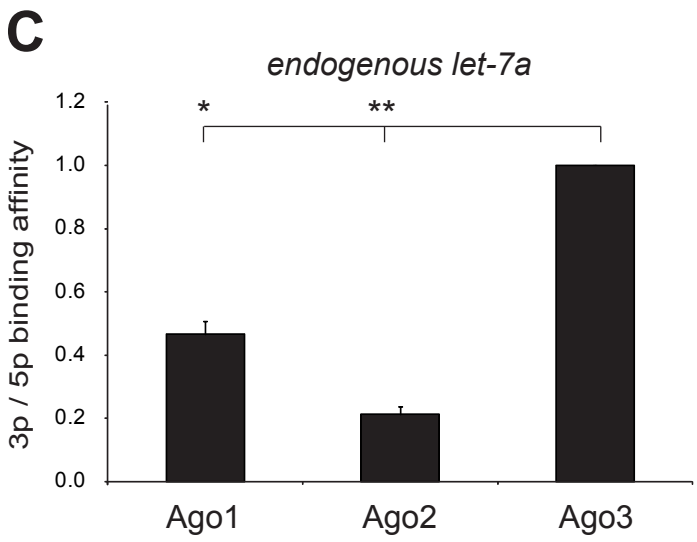
Suppl Fig 11

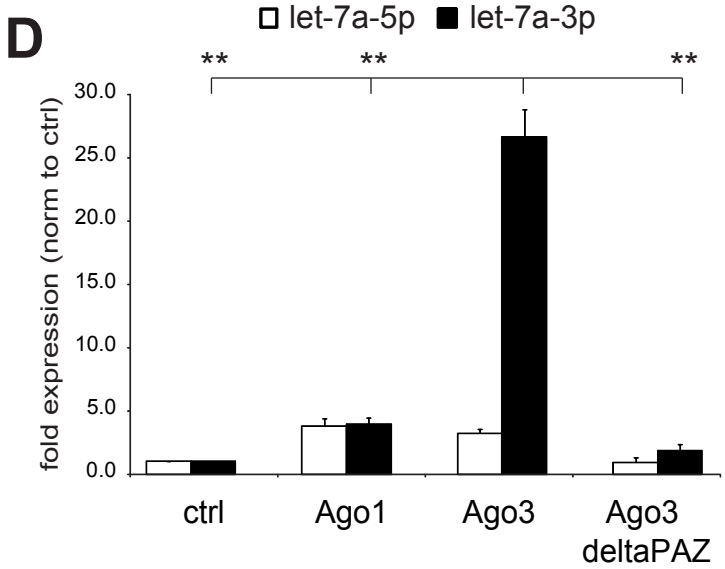

Ago1
Ago3
N
PAZ
Mid
PIWI
miR-20a-5p
miR-20a-3p
ctrl
Ago1 WT
Ago3 WT
Ago m#1
Ago m#2
Ago m#3
Ago m#4
Ago m#5
Ago m#6
Ago m#7
0.0
2.0
4.0
6.0
8.0
10.0
fold expression (norm to ctrl)

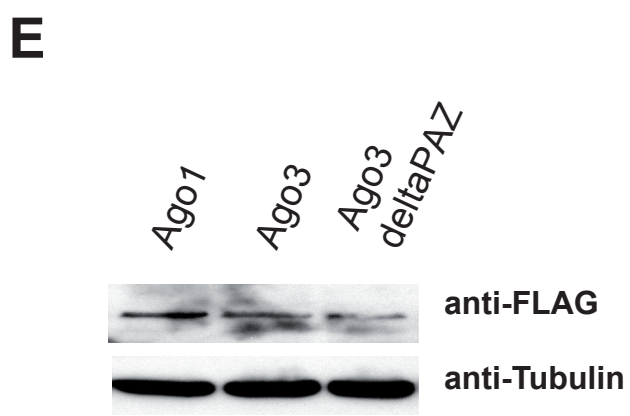
Suppl Fig 12


A
Ago FLAG
tfx
let-7a
FLAG-beads
block w/ BSA & tRNA
lyse
wash
Ago FLAG
wash
RNA isolation
detect miRNA expression via qPCR


B
ectopic let-7a
3p / 5p binding affinity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Ago1
Ago2
Ago3

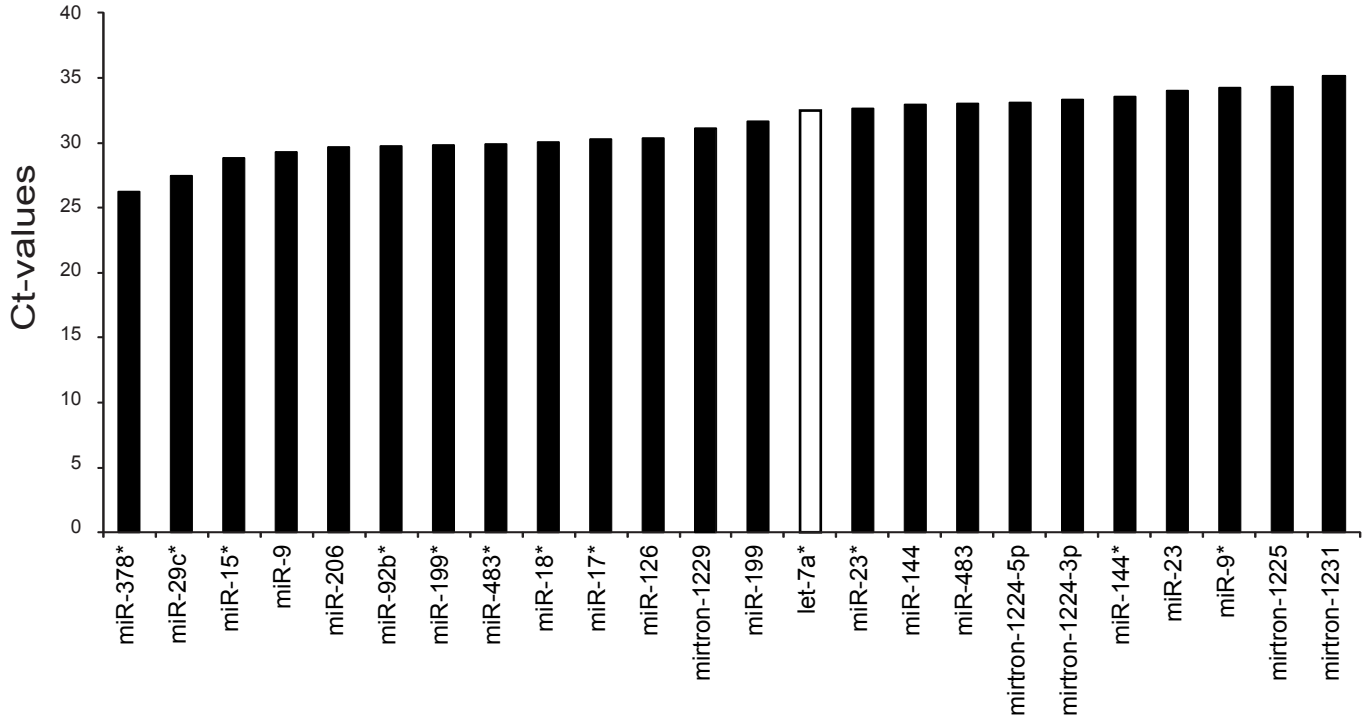

C
endogenous let-7a
3p / 5p binding affinity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
Ago1
Ago2
Ago3


D
let-7a-5p
let-7a-3p
fold expression (norm to ctrl)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
**
**
**
ctrl
Ago1
Ago3
Ago3 deltaPAZ

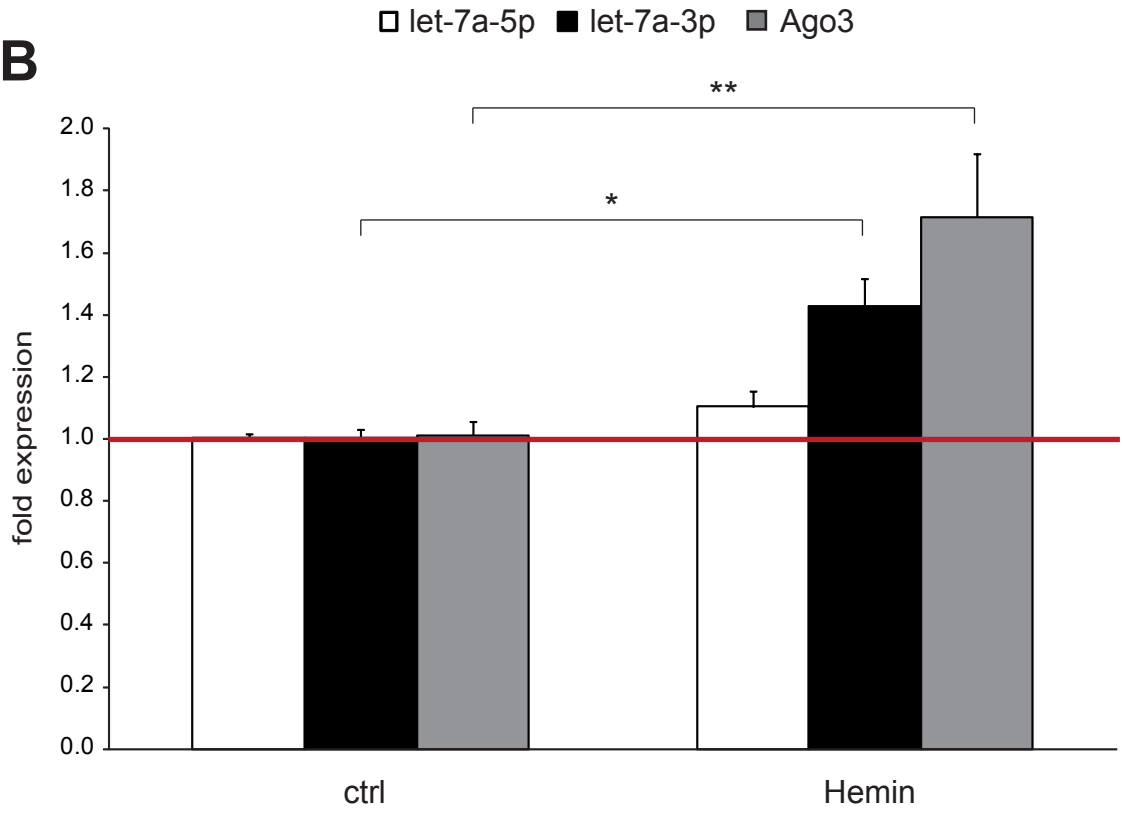

E
Ago1
Ago3
Ago3 deltaPAZ
anti-FLAG
anti-Tubulin

Suppl Fig 13

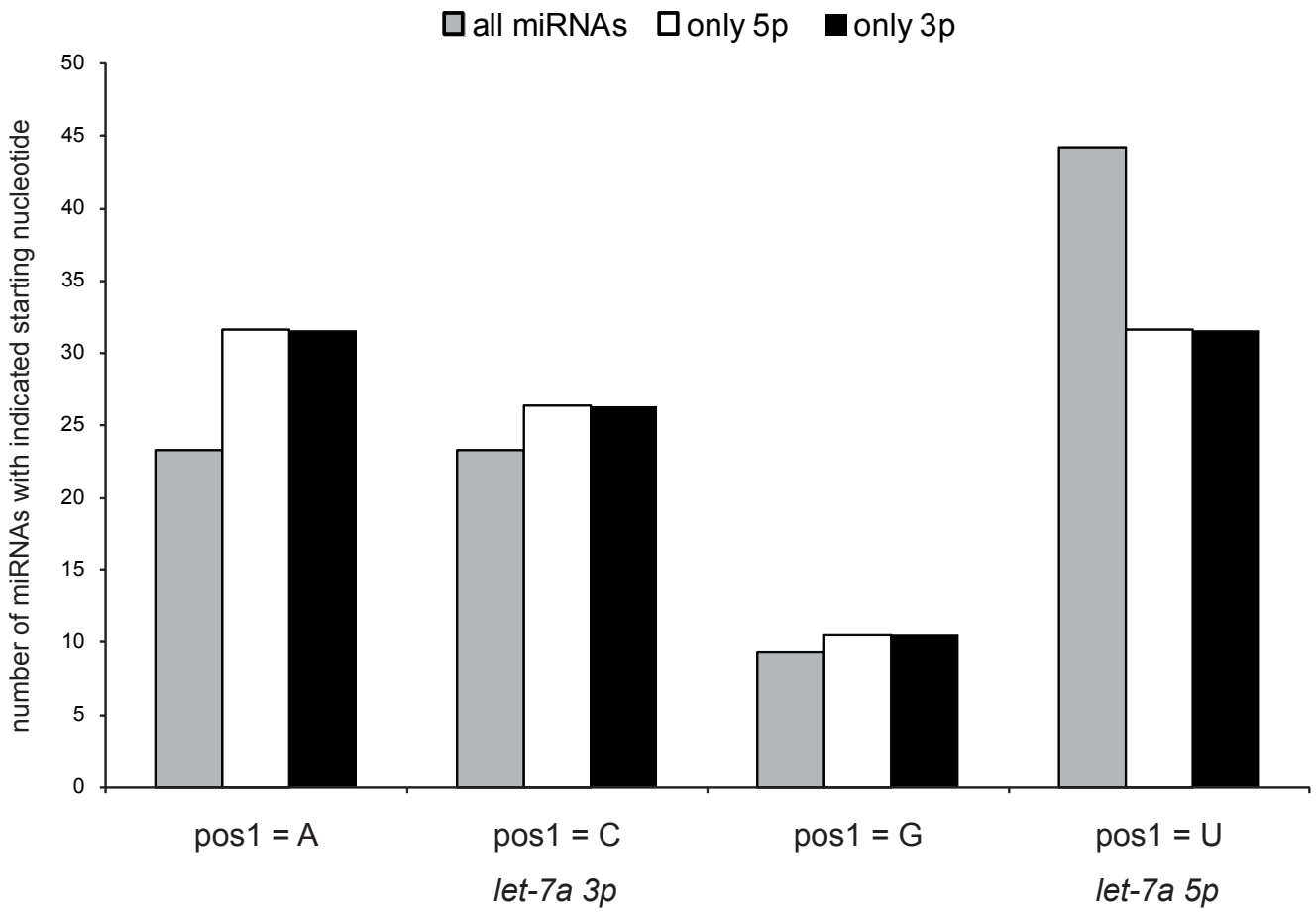
A



B



Suppl Fig 14


all miRNAs
only 5p
only 3p
number of miRNAs with indicated starting nucleotide
50
45
40
35
30
25
20
15
10
5
0
pos1 = A
pos1 = C
let-7a 3p
pos1 = G
pos1 = U
let-7a 5p

Suppl Fig 15

	Sequence	Name
Northern Blot probes	AACTATACAACCTACTACCTCA	let-7a-5p AS NB
	GAAAGACAGTAGATTGTATAG	let-7a-3p AS NB
	TGTGCTGCCGAAGCGAGCAC	U6 snRNA AS NB
Cloning of synthetic luciferase constructs	TCGAGAACTATACAACCTACTACCTCAAACTATACAACCTACTACCTCAAACTATACAACCTACTACCTCA AACTATACAACCTACTACCTCAT	let-7a 4x pm as XhoI XbaI
	CTAGATGAGGTAGTAGGTTGTATAGTT TGAGGTAGTAGGTTGTATAGTTTGAGGTAGTAGGTTGTATAGTTTGAGGTAGTAGGTTGTATAGTTC	let-7a 4x pm s XhoI XbaI
	TCGAGGAAAGACAGTAGATTGTATAGGAAAGACAGTAGATTGTATAGGAAAGACAGTAGATTGTATAGGAAAGACAGTAGATTGTATAGT	let-7a-3-3p 4xwt as XhoI XbaI
	CTAGACTATACAATCTACTGTCTTTCCTATACAATCTACTGTCTTTCCTATACAATCTACTGTCTTTCCTATACAATCTACTGTCTTTCC	let-7a-3-3p 4xwt s XhoI XbaI
	TCGAGGAAAGACAGTTCATTGTATAGGAAAGACAGTTCATTGTATAGGAAAGACAGTTCATTGTATAGGAAAGACAGTTCATTGTATAGT	let-7a-3-3p 4xMM10&11 as XhoI XbaI
	CTAGACTATACAATGAACTGTCTTTCCTATACAATGAACTGTCTTTCCTATACAATGAACTGTCTTTCCTATACAATGAACTGTCTTTCC	let-7a-3-3p 4xMM10&11 s XhoI XbaI
	TCGAGGAAAGACAGTAGATTCAATAGGAAAGACAGTAGATTCAATAGGAAAGACAGTAGATTCAATAGGAAAGACAGTAGATTCAATAGT	let-7a-3-3p 4xMM5&6 as XhoI XbaI
	CTAGACTATTGAATCTACTGTCTTTCCTATTGAATCTACTGTCTTTCCTATTGAATCTACTGTCTTTCCTATTGAATCTACTGTCTTTCC	let-7a-3-3p 4xMM5&6 s XhoI XbaI
Cloning primer	TCTCCTGTTCCATCAGTTGC	RAB10 3´UTR F
	AGAATATGAAACCGAATTTGTAGC	RAB10 3´UTR R
	ATCTATTCTGCAAATCGCATAAGAATAAAGTTAGA	RAB10 3´UTR mut F#1
	TCTAACTTTATTCTTATGCGATTTGCAGAATAGAT	RAB10 3´UTR mut R#1
	GTATAAATCAATTTTCGCATAATCTGTTTGAAACA	RAB10 3´UTR mut F#2
	TGTTTCAAACAGATTATGCGAAAATTGATTTATAC	RAB10 3´UTR mut R#2
	CACCGAGCGTCGTGTAACCCTTGG	miR-193a TOPO F
	GCACCTCACCACTCCTTCTCC	miR-193a TOPO R
	GAGGTAGTAGGTTGTATAGTTGGGTGTCGGATCCTATACAATCTACTGTCTTTC	let-7a-3 + loop miR-193a F
	GAAAGACAGTAGATTGTATAGGATCCGACACCCAACTATACAACCTACTACCTC	let-7a-3 + loop miR-193a R
	CTTTGCGGGCGAGATGATGGGGCTCTGCCCTGCTATGGGATAAAACTGGCCTACAAAGTC	miR-193a + loop let-7a F
	GACTTTGTAGGCCAGTTTTATCCCATAGCAGGGCAGAGCCCCATCATCTCGCCCGCAAAG	miR-193a + loop let-7a R
	ACCAGCTGGGGCTATAGTGCATTCTAGT	Ago mut to remove XhoI restriction site F
	ACTAGAATGCACTATAGCCCCAGCTGGT	Ago mut to remove XhoI restriction site R
	CACGGACTCTCGAGAGCGCGTTCGC	Ago1 Xho1 N-term mut F
	GCGAACGCGCTCTCGAGAGTCCGTG	Ago1 Xho1 N-term mut R
	TCTGACTGATTCTCGAGATCGGGTAAAATTC	Ago3 Xho1 N-term mut F
	GAATTTTACCCGATCTCGAGAATCAGTCAGA	Ago3 Xho1 N-term mut R
	CAGACAGACAGCTCGAGGAGATCAGT	Ago1 Xho1 N+PAZ mut F
	ACTGATCTCCTCGAGCTGTCTGTCTG	Ago1 Xho1 N+PAZ mut R
	TAGACAACTCGAGGAAATTAGC	Ago3 Xho1 N+PAZ mut F
	GCTAATTTCCTCGAGTTGTCTA	Ago3 Xho1 N+PAZ mut R
	ACAGACTGTGCTCGAGTGCACAGT	Ago1 Xho1 MID+PIWI mut F
	ACTGTGCACTCGAGCACAGTCTGT	Ago1 Xho1 MID+PIWI mut R
	GCCAAACTGTGCTCGAGAGAACAGTA	Ago3 Xho1 MID+PIWI mut F
	TACTGTTCTCTCGAGCACAGTTTGG	Ago3 Xho1 MID+PIWI mut R
	ATGTTCCGGCATCTCGAGAAGAACACCTAC	Ago1 Xho1 PIWI mut F
	GTAGGTGTTCTTCTCGAGATGCCGGAACAT	Ago1 Xho1 PIWI mut R
	ATGTTCCGGCATCTCGAGAAGAACACATAT	Ago3 Xho1 PIWI mut F
	ATATGTGTTCTTCTCGAGATGCCGGAACAT	Ago3 Xho1 PIWI mut R
qPCR primer	ACCAAGACCGACTGCCCTTT	pri-hsa-let-7a-3 F
	CTCTGTCCACCGCAGATATT	pri-hsa-let-7a-3 R
	CCCTAAGATCGACGTGTACCACTAC	Ago1 F
	ACCACTTCCCGGTTGACTCTAC	Ago1 R
	CCGGCCTTCTCTCTGGAAAA	Ago2 F
	GCCTTGTAAAACGCTGTTGCT	Ago2 R
	TGTGGACACGAAAATCACC	Ago2 F
	GTGAGCGTAGTATGCTGGC	Ago2 R
	CTCTGACTGATTCTCATCGGGTAA	Ago3 F
	CACAATGAGTCACTTCAACCTTCAA	Ago3 R
	AGTTGCTTGTTTTGCACCTCAGA	Ago4 F
	ATTTTACGCAGCTGGTCAGTGA	Ago4 R
	GCCCGTCGTACCGTGAGTAATAA	Hsa-mir-126 sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCATT	Hsa-mir-126 sl RT
	GCCCGCATTATTACTTTTGGTACGCG	Hsa-mir-126* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCGTA	Hsa-mir-126* sl RT
	GCCCGTACAGTATAGATGATGTA	Hsa-mir-144 sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGTACA	Hsa-mir-144 sl RT
	GCCCGGGATATCATCATATACTG	Hsa-mir-144* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTTACA	Hsa-mir-144* sl RT
	GCCCGCAAAGTGCTTACAGTGCA	Hsa-mir-17a sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTACCT	Hsa-mir-17a sl RT
	GCCCGACTGCAGTGAAGGCACTT	Hsa-mir-17a* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTACAA	Hsa-mir-17a* sl RT
	GCCCGTAAGGTGCATCTAGTGCA	Hsa-mir-18a sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTATCT	Hsa-mir-18a sl RT
	GCCCGACTGCCCTAAGTGCTCCT	Hsa-mir-18a* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCAGAA	Hsa-mir-18a* sl RT
	GCCCGAACTGGCCTACAAAGTCC	Hsa-miR-193a-3p sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTGGG	Hsa-miR-193a-3p sl RT
	GCCCGTGGGTCTTTGCGGGCGAG	Hsa-miR-193a-5p sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCATCT	Hsa-miR-193a-5p sl RT
	GCCCGCCCAGTGTTCAGACTACC	Hsa-mir-199a sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAACAG	Hsa-mir-199a sl RT
	GCCCGACAGTAGTCTGCACATTG	Hsa-mir-199a* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTAACCA	Hsa-mir-199a* sl RT
	GCCCGATCACATTGCCAGGGATT	Hsa-mir-23b sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGTAAT	Hsa-mir-23b sl RT
	GCCCGTGGGTTCCTGGCATGCTG	Hsa-mir-23b* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAATCA	Hsa-mir-23b* sl RT
	GCCCGTAGCACCATTTGAAATCG	Hsa-mir-29c sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTAACCG	Hsa-mir-29c sl RT
	GCCCG TGACCGATTTCTCCTGGT	Hsa-mir-29c* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAACAC	Hsa-mir-29c* sl RT

	GCCCGCAGTGCCTCGGCAGTGCA	Hsa-miR-33b sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGGCTG	Hsa-miR-33b sl RT
	GCCCGCAGTGCCTCGGCAGTGCA	Hsa-miR-33b* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGGCTG	Hsa-miR-33b* sl RT
	GCCCGACTGGACTTGGAGTCAGA	Hsa-mir-378 sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCTTCT	Hsa-mir-378 sl RT
	GCCCGCTCCTGACTCCAGGTCCT	Hsa-mir-378* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACACAG	Hsa-mir-378* sl RT
	GCCCGGCAGTCCATGGGCATATA	Hsa-miR-455* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTGTAT	Hsa-miR-455* sl RT
	GCCCGAAGACGGGAGGAAAGAAG	Hsa-mir-483 sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTCCCT	Hsa-mir-483 sl RT
	GCCCGTCACTCCTCTCCTCCCGT	Hsa-mir-483* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAGACG	Hsa-mir-483* sl RT
	GCCCGCGTCAACACTTGCTGGTT	Hsa-miR-505 sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGAAA	Hsa-miR-505 sl RT
	GCCCG GTTCTCCCAACGTAAGCC	Hsa-mir-629-3p sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCTGGG	Hsa-mir-629-3p sl RT
	GCCCGTGGGTTTACGTTGGGAGA	Hsa-mir-629-5p sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGTTCT	Hsa-mir-629-5p sl RT
	GCCCGTCTTTGGTTATCTAGCTG	Hsa-mir-9 sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCATAC	Hsa-mir-9 sl RT
	GCCCGATAAAGCTAGATAACCGA	Hsa-mir-9* sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTTTC	Hsa-mir-9* sl RT
	GCCCGAGGGACGGGACGCGGTGC	Hsa-mir-92b-3p sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACTGC	Hsa-mir-92b-3p sl RT
	GCCCGTATTGCACTCGTCCCGGC	Hsa-mir-92b-5p sl F
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGAGGC	Hsa-mir-92b-5p sl RT
siRNA	GGTAAGAAGTGCAAATTAT	si_Ago3#1
	GCGTACTTCCAGCACCTAT	si_Ago3#2

Supplementary Table 1: Oligonucleotide sequences

All oligonucleotide sequences are given in 5'-3' direction.

Figure	condition		norm to	p-value
Figure 1C	let-7a-3p	ctrl	Ago3	0.024
		Ago1	Ago3	0.026
		Ago2	Ago3	0.031
		Ago4	Ago3	0.028
	let-7a-5p /-3p ratio	ctrl	Ago3	0.018
		Ago1	Ago3	0.005
		Ago2	Ago3	0.018
		Ago4	Ago3	0.014
Figure 2C	let-7a-3p	ctrl	Ago3	<0.001
		Ago1	Ago3	0.029
		Ago2	Ago3	<0.001
		Ago4	Ago3	0.007
	let-7a-5p /-3p ratio	ctrl	Ago3	0.007
		Ago1	Ago3	0.008
		Ago2	Ago3	0.040
		Ago4	Ago3	0.039
Figure 2D	let-7a-3p	ctrl	Ago3	0.022
		Ago1	Ago3	0.017
		Ago2	Ago3	0.023
		Ago4	Ago3	0.025
	let-7a-5p /-3p ratio	ctrl	Ago3	0.007
		Ago1	Ago3	0.023
		Ago2	Ago3	0.020
		Ago4	Ago3	0.025
Figure 3A	Ago3	siAgo3#1	sictrl	0.001
	Ago3	siAgo3#2	sictrl	0.001
Figure 3B	let-7a-3p	siAgo3#1	sictrl	<0.001
	let-7a-3p	siAgo3#2	sictrl	<0.001
Figure 4B	let-7a-3p	ctrl	Ago3	<0.001
	let-7a-3p	Ago1	Ago3	0.002
	let-7a-3p	Ago2	Ago3	0.004
	let-7a-3p	Ago4	Ago3	<0.001
	let-7a-3p /-5p ratio	ctrl	Ago3	<0.001
	let-7a-3p /-5p ratio	Ago1	Ago3	0.016
	let-7a-3p /-5p ratio	Ago2	Ago3	<0.001
	let-7a-3p /-5p ratio	Ago4	Ago3	<0.001
Figure 4C	let-7a-3p	ctrl	Ago3	<0.001
	let-7a-3p	Ago1	Ago3	0.009
	let-7a-3p	Ago2	Ago3	0.014
	let-7a-3p	Ago4	Ago3	<0.001
	let-7a-3p /-5p ratio	ctrl	Ago3	0.004
	let-7a-3p /-5p ratio	Ago1	Ago3	0.008
	let-7a-3p /-5p ratio	Ago2	Ago3	0.003
	let-7a-3p /-5p ratio	Ago4	Ago3	<0.001
Figure 6	let-7a-3p	Ago3 WT	Ago1	0.024
	let-7a-3p	Ago m#2	Ago1	0.038
	let-7a-3p	Ago m#5	Ago1	0.009
	let-7a-3p	Ago m#7	Ago1	0.003

Suppl. Fig. 1	let-7a-3p	ctrl	Ago3	0.027
		Ago1	Ago3	0.027
		Ago2	Ago3	0.029
		Ago4	Ago3	0.028
Suppl. Fig. 5	RAB10	let-7a + Ago3	let-7a	0.050
Suppl. Fig. 6E	let-7a-3p	ctrl	Ago3	0.032
		Ago1	Ago3	0.040
Suppl. Fig. 7B	let-7a-3p	ctrl	Ago3	0.012
		Ago1	Ago3	0.013
		Ago2	Ago3	0.015
		Ago4	Ago3	0.018
	let-7a-5p /-3p ratio	ctrl	Ago3	0.034
		Ago1	Ago3	0.018
		Ago2	Ago3	0.025
		Ago4	Ago3	0.032
Suppl. Fig. 7D	let-7a-3p	ctrl	Ago3	0.012
		Ago1	Ago3	0.002
		Ago2	Ago3	0.004
		Ago4	Ago3	0.032
	let-7a-5p /-3p ratio	ctrl	Ago3	<0.001
		Ago1	Ago3	0.012
		Ago2	Ago3	0.008
		Ago4	Ago3	0.034
Suppl. Fig. 8	let-7a-5p	Ago2-KO	WT	0.013
Suppl. Fig. 10	let-7a-3p (WT)	ctrl	Ago3	0.039
		Ago1	Ago3	0.042
		Ago2	Ago3	0.012
	let-7a-3p (MM10)	Ago2	Ago3	0.038
	let-7a-3p (MM11)	ctrl	Ago3	0.019
		Ago1	Ago3	0.004
		Ago2	Ago3	0.010
	let-7a-3p (MM10&11)	ctrl	Ago3	0.001
		Ago1	Ago3	0.001
Suppl. Fig. 13B	let-7a-3p /-5p binding affinity	Ago2	Ago3	0.02
Suppl. Fig. 13C	let-7a-3p /-5p binding affinity	Ago1	Ago3	0.012
	let-7a-3p /-5p binding affinity	Ago2	Ago3	0.002
Suppl. Fig. 13D	let-7a-3p	GFP	Ago3	0.007
	let-7a-3p	Ago1	Ago3	0.007
	let-7a-3p	Ago3 delta PAZ	Ago3	0.006
Suppl. Fig. 14B	Hemin	let-7a 3p	ctrl	0.011
	Hemin	Ago3	ctrl	0.005

Supplementary Table 2: p-values of statistical analyses (t-tests)