SUPPLEMENTAL FIGURES AND LEGENDS

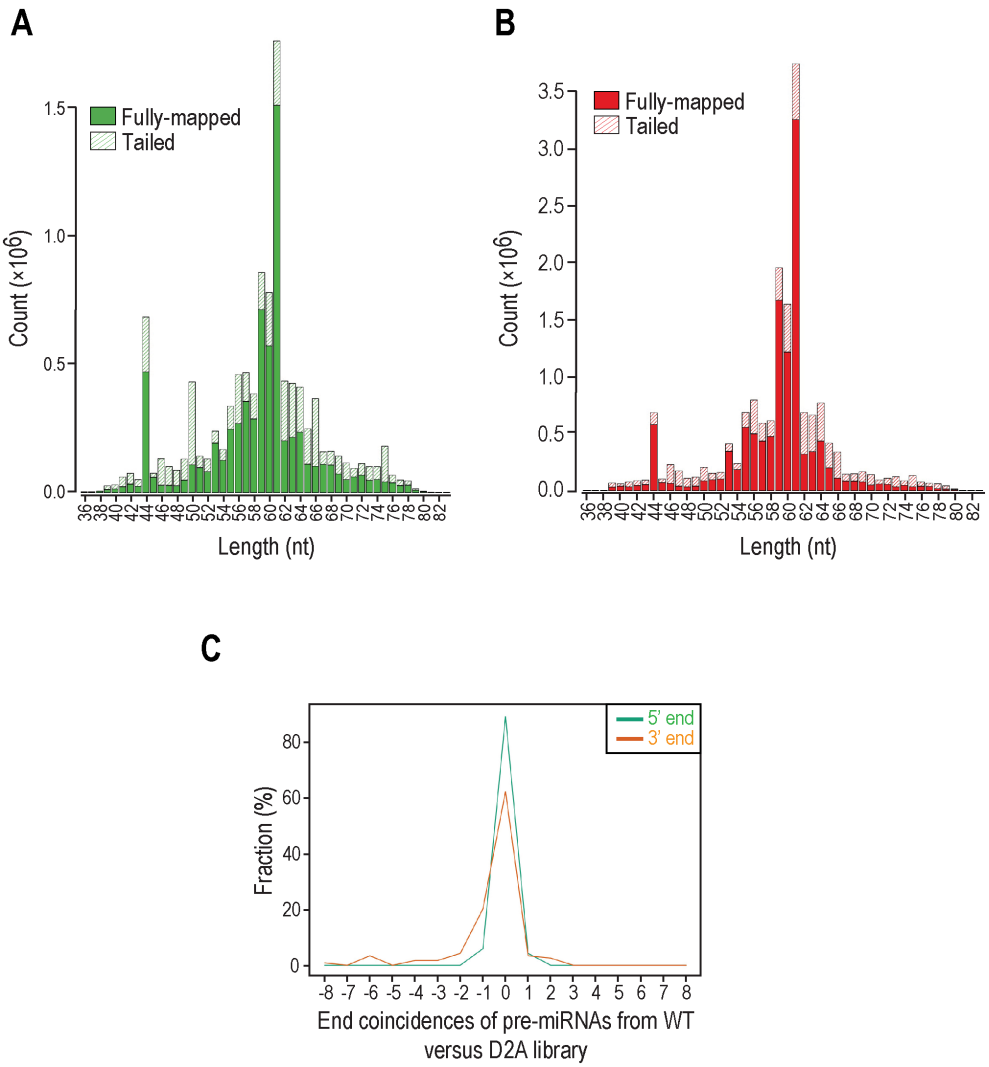


Figure S1. Related to Figure 1

Genome-wide profiling of Ago-bound, full-length precursor miRNAs

A, B. Length distribution of fully-mapped or tailed reads in *WT* (**A**) or *D2A* (**B**) pre-miRNA libraries.

C. Coincidence of defined 5' or 3' ends of pre-miRNAs between *WT* and *D2A* pre-miRNA libraries. Positive and negative values indicate that the defined ends of pre-miRNAs from the *WT* library are beyond and within those from the *D2A* library respectively.

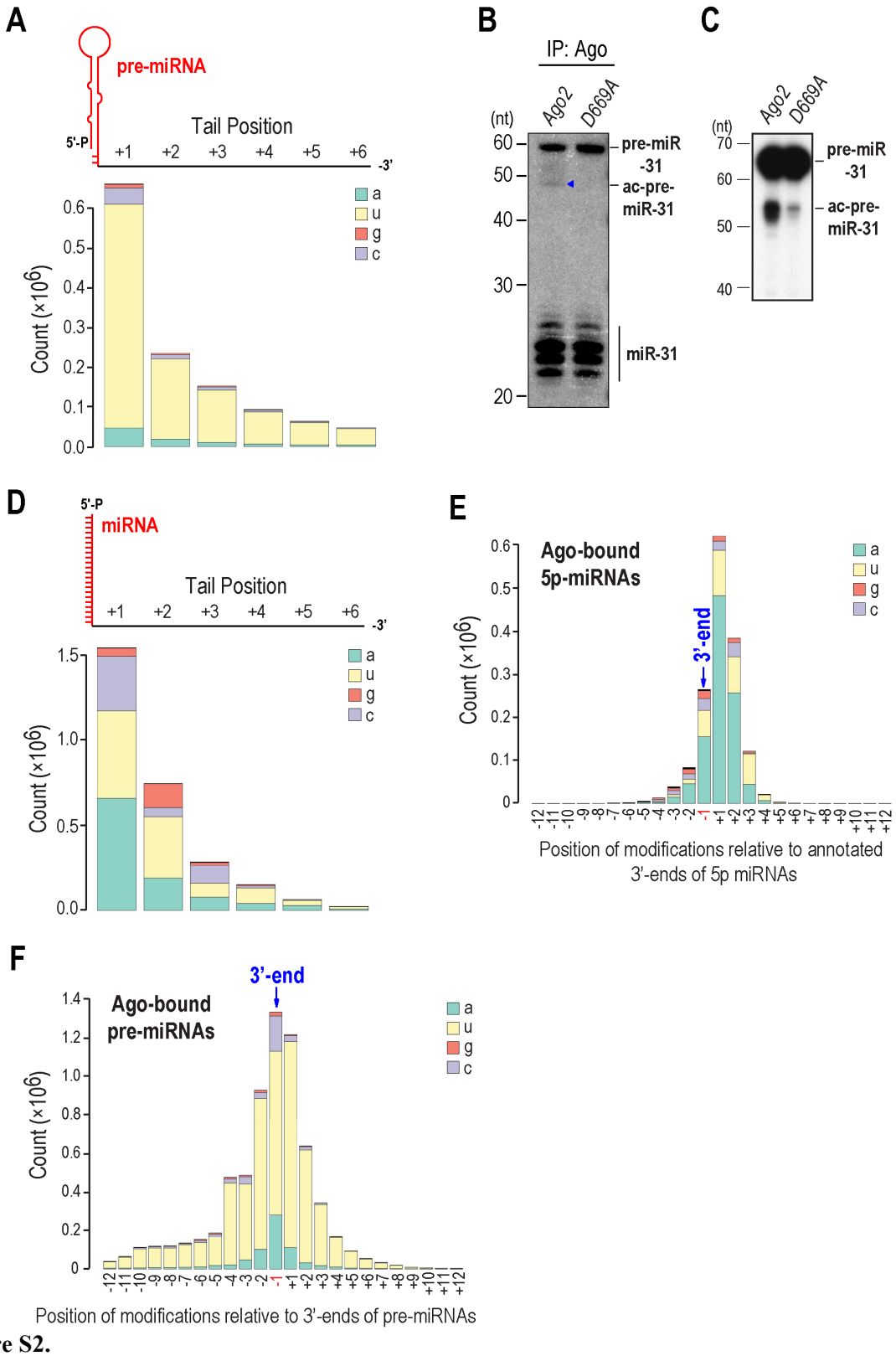

A
pre-miRNA
5'-P
Tail Position
+1 +2 +3 +4 +5 +6
-3'
Count (×10^6)
a
u
g
c
B
IP: Ago
Ago2
D669A
(nt)
pre-miR-31
ac-pre-miR-31
miR-31
C
Ago2
D669A
(nt)
pre-miR-31
ac-pre-miR-31
D
5'-P
miRNA
Tail Position
+1 +2 +3 +4 +5 +6
-3'
Count (×10^6)
E
Ago-bound 5p-miRNAs
3'-end
Count (×10^6)
Position of modifications relative to annotated 3'-ends of 5p miRNAs
F
Ago-bound pre-miRNAs
3'-end
Count (×10^6)
Position of modifications relative to 3'-ends of pre-miRNAs

Figure S2.

Figure S2. Related to Figure 2

Widespread and extensive post-transcriptional modifications in Ago-bound pre-miRNAs

A. Nucleotide composition of RNA modifications for every tail position in *WT* pre-miRNA library.

B. Northern blot for miR-31 from Ago IPs from *wild-type* (*Ago2*+) or catalytically inactive (*D669A*+) *Ago2*-transduced MEFs. Ac-pre-miR-31: Ago2-cleaved pre-miR-31.

C. *In vitro* cleavage assay. 5' radiolabeled pre-miR-31 was incubated in lysates prepared from *Ago2*+ or *D669A*+ MEFs.

D. Nucleotide composition of RNA modifications for every tail position in *WT* miRNA library.

E. Nucleotide composition of RNA modifications surrounding the annotated 5' ends of 5p miRNAs in *WT* miRNA library.

F. Nucleotide composition of RNA modifications surrounding the defined 3' ends of pre-miRNAs in *D2A* pre-miRNA library.

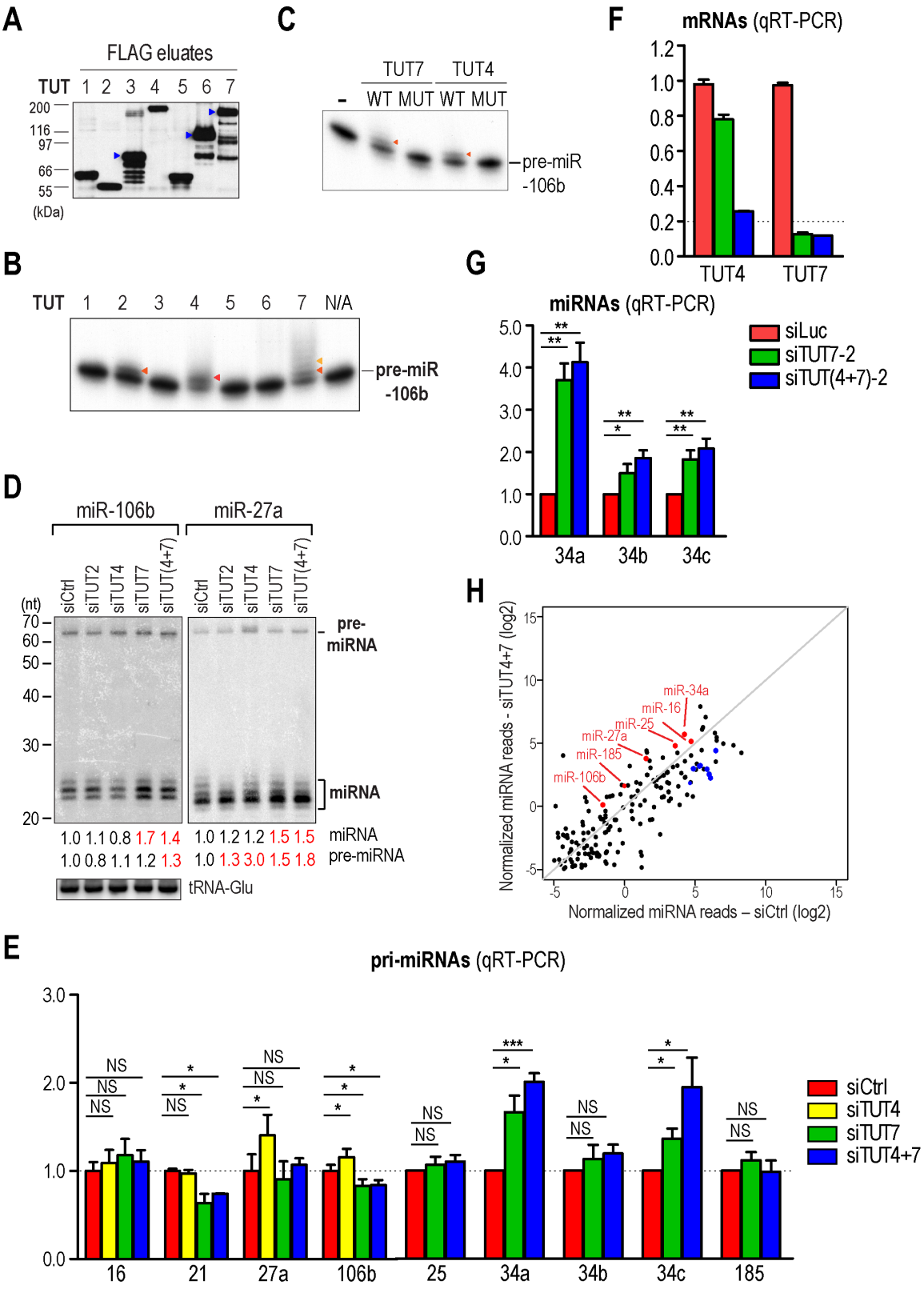

A
FLAG eluates
TUT 1 2 3 4 5 6 7
200
116
97
66
55
(kDa)
C
TUT7 TUT4
- WT MUT WT MUT
pre-miR
-106b
F
mRNAs (qRT-PCR)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
TUT4
TUT7
B
TUT 1 2 3 4 5 6 7 N/A
pre-miR
-106b
G
miRNAs (qRT-PCR)
siLuc
siTUT7-2
siTUT(4+7)-2
5.0
4.0
3.0
2.0
1.0
0.0
34a
34b
34c
D
miR-106b
miR-27a
siCtrl
siTUT2
siTUT4
siTUT7
siTUT(4+7)
(nt)
70
60
50
40
30
20
pre-
miRNA
miRNA
1.0 1.1 0.8 1.7 1.4
1.0 1.2 1.2 1.5 1.5
miRNA
1.0 0.8 1.1 1.2 1.3
1.0 1.3 3.0 1.5 1.8
pre-miRNA
tRNA-Glu
H
Normalized miRNA reads - siTUT4+7 (log2)
Normalized miRNA reads – siCtrl (log2)
miR-34a
miR-16
miR-25
miR-27a
miR-185
miR-106b
E
pri-miRNAs (qRT-PCR)
3.0
2.0
1.0
0.0
siCtrl
siTUT4
siTUT7
siTUT4+7
16
21
27a
106b
25
34a
34b
34c
185

Figure S3.

Figure S3. Related to Figure 3

Uridylation of pre-miRNAs by TUT7 and TUT4 fine tunes select miRNA biogenesis

A. Western blot of eluates from immunopurified FLAG-TUTs: TUT1 (mtPAP/PAPD1), TUT2 (GLD2/PAPD4), TUT3 (PAPD5/TRF4-2), TUT4 (ZCCHC11/PAPD3), TUT5 (POLS/TRF4-1), TUT6 (PAPD2) and TUT7 (ZCCHC6/PAPD6).

B. Uridylation assay of 5' radiolabeled pre-miR-106b with indicated FLAG-TUTs.

C. Uridylation assay of pre-miR-106b with wild-type (WT) or catalytically inactive (Mut) FLAG-TUT4 and FLAG-TUT7.

D. Representative northern blots for miR-106b and miR-27a from indicated knockdown cells. Numbers indicate fold changes of pre-miRNAs and miRNAs relative to the control knockdown. tRNA-Glu: loading control.

E. Pri-miRNA levels in indicated knockdown cells, measured by northern blotting. standard error of the mean (SEM) is from at least three independent experiments. Statistical significance was calculated by two-tail t test.

F, G. TUT4 and TUT7 (**F**) as well as miRNA (**G**) levels in MEFs depleted with a second set of siRNAs against TUT4 and TUT7, measured by quantitative real-time (qRT)-PCR (**F**) and Taqman miRNA assay (**G**) respectively. SEM (Werner et al. 2008) is from three experiments. siLuc, siRNA against Luciferase. Statistical significance was calculated by one-tail t test.

H. The abundance of a subset of miRNAs is significantly altered upon depletion of TUT4 and TUT7. Red dots designate miRNAs that have been experimentally verified in this study. Blue dots denote let-7 family members.

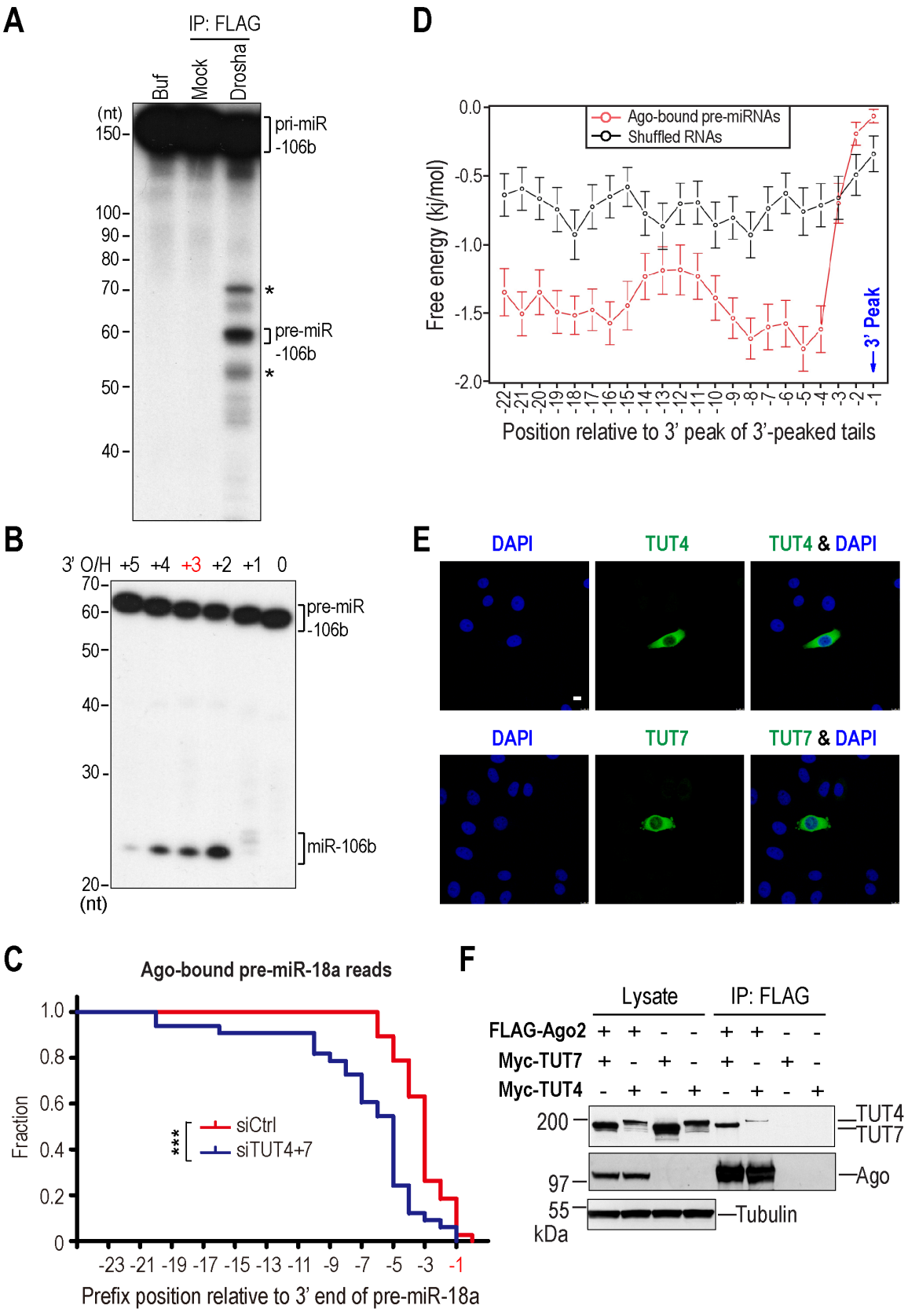

A
IP: FLAG
Buf
Mock
Drosha
(nt)
150
100
90
80
70
60
50
40
pri-miR
-106b
*
pre-miR
-106b
*
B
3' O/H +5 +4 +3 +2 +1 0
70
60
50
40
30
20
(nt)
pre-miR
-106b
miR-106b
C
Ago-bound pre-miR-18a reads
1.0
0.8
0.6
0.4
0.2
0
Fraction
***
siCtrl
siTUT4+7
-23 -21 -19 -17 -15 -13 -11 -9 -7 -5 -3 -1
Prefix position relative to 3' end of pre-miR-18a
D
Ago-bound pre-miRNAs
Shuffled RNAs
0.0
-0.5
-1.0
-1.5
-2.0
Free energy (kj/mol)
3' Peak
-22 -21 -20 -19 -18 -17 -16 -15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1
Position relative to 3' peak of 3'-peaked tails
E
DAPI
TUT4
TUT4 & DAPI
DAPI
TUT7
TUT7 & DAPI
F
Lysate
IP: FLAG
FLAG-Ago2 + + - - + + - -
Myc-TUT7 + - + - + - + -
Myc-TUT4 - + - + - + - +
200
97
55
kDa
TUT4
TUT7
Ago
Tubulin

Figure S4.

Figure S4. Related to Figure 4

Uridylation of Ago-bound pre-miRNAs by TUT7 and TUT4 facilitates their degradation

A. In-vitro Drosha processing of pri-miR-106b. Asterisks depict single strands that flank the stem of the hairpin.

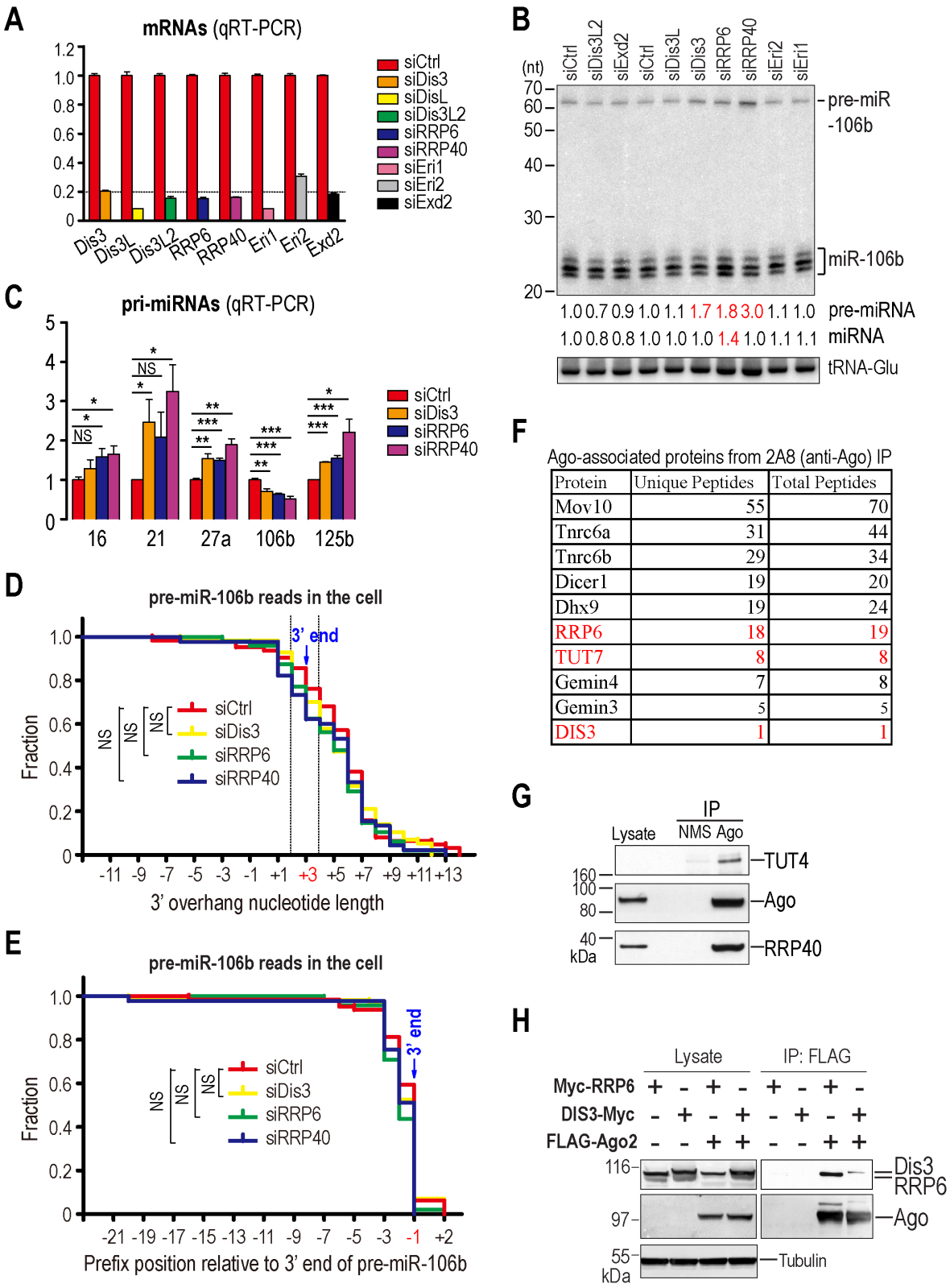
B. In-vitro Dicer processing of pre-miR-106b with a native or progressively shortened 3' overhangs.

C. Reverse cumulative plot of prefix positions of pre-miR-18a reads relative to its native 3' end (position -1), sequenced from Ago-IPs from indicated knockdown cells. Statistical significance was calculated by Anderson-Darling (A-D) test. NS, non-significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Same depiction applies to all figures.

D. Average Gibbs free energy for every position upstream of the 3' peak of tailed Ago-bound pre-miRNAs or of randomly shuffled RNAs. Error bars represent 95% CIs.

E. Subcellular localization of TUT4 and TUT7. Representative confocal images of HeLa cells transfected with FLAG-TUT4 or FLAG-TUT7. Blue, DAPI; Green, FLAG-TUT4/7. Scale bar, 10 μm.

F. Interaction of TUT4 and TUT7 with Ago2. Western blots of lysates and FLAG IPs from 293T cells co-expressing Myc-TUT4 or Myc-TUT7 with FLAG-Ago2. Tubulin: loading control.



Protein	Unique Peptides	Total Peptides
Mov10	55	70
Tnrc6a	31	44
Tnrc6b	29	34
Dicer1	19	20
Dhx9	19	24
RRP6	18	19
TUT7	8	8
Gemin4	7	8
Gemin3	5	5
DIS3	1	1

Figure S5.

Figure S5. Related to Figure 5

Quality control and turnover of pre-miRNAs by the exosome

A. mRNA levels of 3' exoribonucleases in indicated knockdown cells, measured by qRT-PCR. SEM is from four experiments.

B. A representative northern blot of total RNAs from indicated knockdown cells. Numbers indicate fold changes of pre-miR-106b and miR-106b relative to the control knockdown.

C. Pri-miRNA levels from cells in indicated knockdown cells, measured by qRT-PCR. SEM is from four experiments.

D. Reverse cumulative plot of 3' overhang lengths of pre-miR-106b reads (fully-mapped and tailed), sequenced from indicated knockdown cells. Reads whose ends fall within the two dotted lines are optimal Dicer substrates. The native 3' overhang length of pre-miR-106b is +3. Positive and negative values indicate 3' and 5' overhangs respectively. Statistical significance was calculated by A-D test.

E. Reverse cumulative plot of prefix positions of pre-miR-106b reads relative to its native 3' end (position -1), sequenced from indicated knockdown cells. Statistical significance was calculated by A-D test.

F, G. Association of RRP6, DIS3, RRP40, TUT4, TUT7 with endogenous Agos in MEFs. Endogenous Agos were immunoprecipitated with an anti-Ago monoclonal antibody (2A8) in Dicer knock-out MEFs reconstituted with catalytically inactive Dicer (D2A); immunoprecipitates were resolved by NuPAGE and co-precipitating proteins were excised from the gel and identified by mass spectrometry (**F**) or by Western blots (**G**); non-immune mouse serum (NMS) served as negative control. This approach was chosen because in D2A cells the majority of Agos associate with pre-miRNAs in miRLC or miPDC (Liu et al., 2012), which allows isolation of proteins that interact with Ago/pre-miRNA. In contrast, in cells with wild-type Dicer the majority of Agos are engaged with miRNAs in miRNP/RISC while levels of miRLC or miPDC are very low.

H. Western blots of lysates and FLAG IPs from 293T cells co-expressing Myc-RRP6 or DIS3-Myc with FLAG-Ago2. Tubulin: loading control.

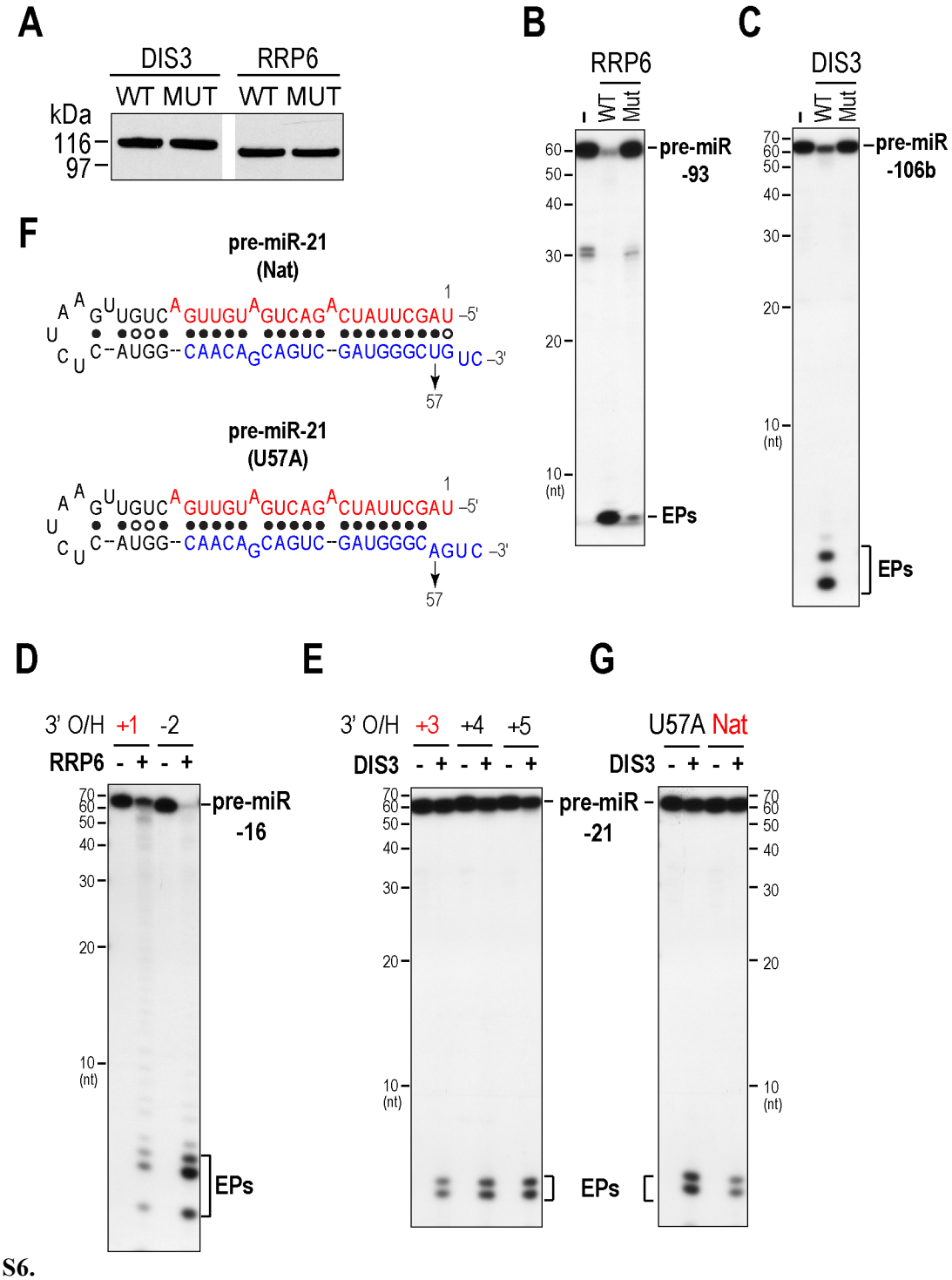

A
DIS3
RRP6
WT MUT
WT MUT
kDa
116
97
B
RRP6
-
WT
Mut
60
50
40
30
20
10
(nt)
pre-miR
-93
EPs
C
DIS3
-
WT
Mut
70
60
50
40
30
20
10
(nt)
pre-miR
-106b
EPs
F
pre-miR-21
(Nat)
AGUUGUCAGUUGUAGUCAGACUAUUCGAU –5'
UCC–AUGG--CAACAGCAGUC–GAUGGGCUGUC –3'
1
57
pre-miR-21
(U57A)
AGUUGUCAGUUGUAGUCAGACUAUUCGAU –5'
UCC–AUGG--CAACAGCAGUC–GAUGGGCAGUC –3'
1
57
D
3' O/H +1 -2
RRP6 - + - +
70
60
50
40
30
20
10
(nt)
pre-miR
-16
EPs
E
3' O/H +3 +4 +5
DIS3 - + - + - +
70
60
50
40
30
20
10
(nt)
pre-miR
-21
EPs
G
U57A Nat
DIS3 - + - +
70
60
50
40
30
20
10
(nt)
EPs

Figure S6.

Figure S6. Related to Figure 6

Distinct preference of DIS3 and RRP6 towards pre-miRNAs

A. Western blots of immunopurified, wild-type (WT) or catalytically inactive (Mut) Dis3-FLAG and FLAG-RRP6.

B. Decay assay using immunopurified, wild-type (WT) or catalytically inactive (Mut) FLAG-RRP6 and 5' radiolabeled pre-miR-93. EPs, end products.

C. Decay assay using immunopurified, wild-type (WT) or catalytically inactive (Mut) DIS3-FLAG and 5' radiolabeled pre-miR-106b.

D. Decay assay using FLAG-RRP6 and pre-miR-16 with either a native 3' overhang or an engineered 2-nt 5' overhang.

E. Decay assay using Dis3-FLAG and pre-miR-21 with either a native 3' overhang or uridylated tails.

F. Schematic of pre-miR-21 and U57A mutant.

G. Decay assay using Dis3-FLAG with native or mutated (U57A) pre-miR-21.

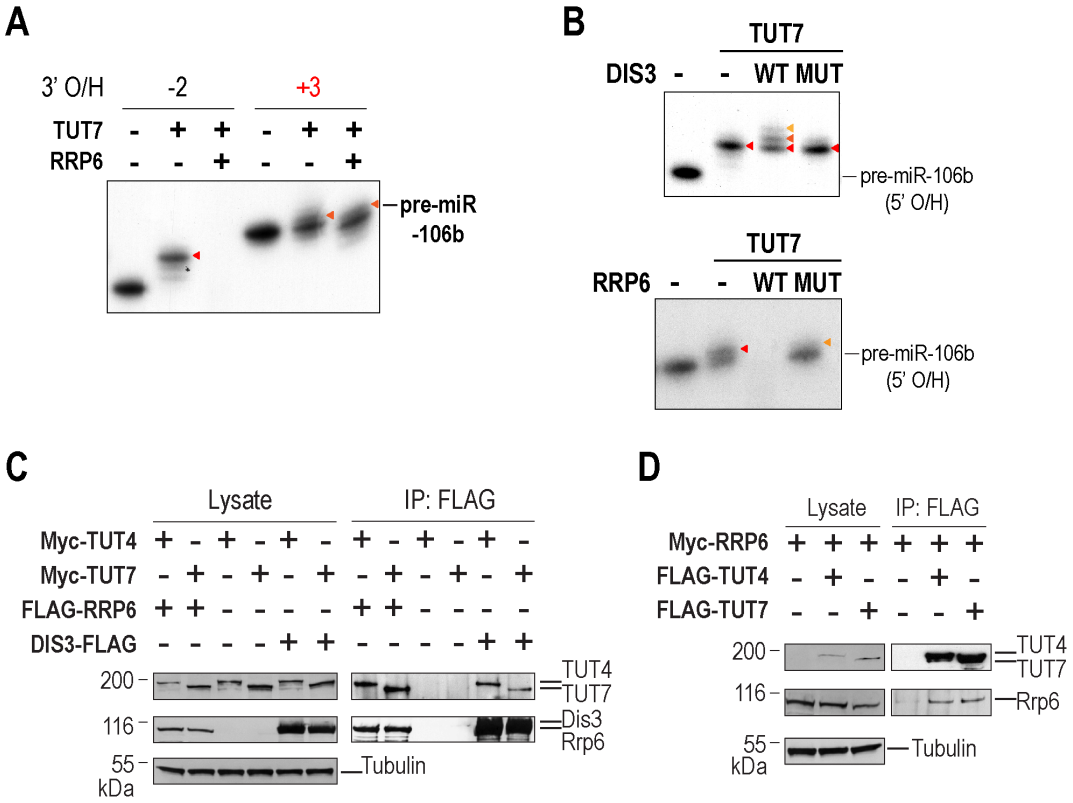


Figure S7. Related to Figure 7

A positive feedback loop constituted by TUT7, TUT4 and the exosome in uridylation and degradation of pre-miRNAs

A. Uridylation assay using FLAG-TUT7 and FLAG-RRP6 with pre-miR-106b harboring different 3' overhangs. The absence of RNAs in lane 3 was due to degradation of pre-miR-106b with 2-nt 5' overhang by FLAG-RRP6 (data not shown).

B. Uridylation assay using FLAG-TUT7 and wild-type (WT) or catalytically inactive (MUT) DIS3 (top panel) or RRP6 (bottom panel) with pre-miR-106b harboring a 2-nt 5' overhang. Catalytically inactive DIS3 or RRP6 do not stimulate the activity of TUT7.

C. Interaction of TUT4 and TUT7 with DIS3 and RRP6. Western blots of lysates and FLAG immunoprecipitates from 293T cells co-expressing Myc-TUT4 or Myc-TUT7 with DIS3-FLAG or FLAG-RRP6. Tubulin: loading control.

D. Interaction of RRP6 with TUT4 and TUT7. Western blots of lysates and FLAG immunoprecipitates from 293T cells co-expressing FLAG-TUT4 or FLAG-TUT7 with Myc-RRP6. Tubulin: loading control.

SUPPLEMENTAL TABLE LEGENDS

Table S1

Profiles of Ago-bound pre-miRNAs from WT or D2A MEFs. Related to Figure 1, 2.

Table S2

Uridylation ratios of 3p miRNAs in knock-down cells, Related to Figure 3.

Table S3

Expression changes of miRNAs in knock-down cells, Related to Figure 3.

Table S4

pre-miR-106b reads from Ago IPs from knock-down MEFs. Related to Figure 4, 5.

Table S5

pre-miR-18a reads from Ago IPs from knock-down MEFs. Related to Figure 4.

Table S6

pre-miR-106b reads from total RNAs of knock-down MEFs. Related to Figure 5.

Table S7

Oligonucleotide sequences used in this study.