Poly(A)-specific ribonuclease sculpts the 3’ ends of microRNAs

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SUPPLEMENTAL MATERIALS AND METHODS

Generation of pre-miRNA substrates with homogenous ends. The exact end structures of pre-miRNAs are critical for DICER processing (Park et al. 2011). Because in vitro transcription using phage RNA polymerases leaves a degree of heterogeneity at both ends of the run-off transcript (Milligan et al. 1987; Krupp 1988), self-cleaving ribozymes were utilized to generate pre-miRNAs with homogenous ends (Schurer et al. 2002; Walker et al. 2003). To this end, the pre-miRNA sequence of interest was fused to a hammerhead (HH) ribozyme and a hepatitis delta virus (HDV) ribozyme at the 5’ and 3’ end, respectively, through two rounds of PCR. The primary structure of the resulting transcription template was then expected to be 5’-[T7 promoter][Reverse complement of the first 11 nt of the pre-miRNA][HH][Pre-miRNA][HDV]-3’. The nucleotide sequences of the HH and HDV ribozymes used here were 5’-CTGATGAGTCCGTGAGGACGAAACGGTACCCGGTACCGTC-3’ and 5’-GGGTCGGCATGGCATCTCCACCTCCTGGGTCGCCACCTGGCCAGAAACTCGGATGGCTAAGGGAGAGCGAGTAGTGGGATCCGGG-3’, respectively. The sequences of oligonucleotides used for ribozyme construction are listed in Table 1. The templates were transcribed in vitro using T7 RNA polymerase at 37°C for 1-3 hr. Following in vitro transcription, the reactions were further subjected to 3 cycles of incubation at 72°C for 1 min, at 65°C for 5 min, then at 37°C for 10 min to ensure ribozyme cleavage. The released pre-miRNA fragments were gel-purified on a 6% urea-polyacrylamide gel. To heal 2’,3’-cyclic phosphates, an undesirable remnant of ribozyme-catalyzed cleavage, the pre-miRNAs were treated with T4 Polynucleotide Kinase (Takara) in home-made Dephosphorylation Buffer (100 mM MES-NaOH at pH 5.5, 10 mM MgCl2, 10 mM βME, and 300 mM NaCl) at 37°C for 6 hr and purified by phenol extraction and ethanol precipitation. The pre-miRNAs were then phosphorylated at the 5’ ends by standard kinasing reactions using T4 Polynucleotide Kinase, gel-purified on a 6% urea-polyacrylamide gel, and used as substrates for in vitro DICER processing. For body-labeling of substrates, the initial transcription reactions were supplemented with [α-32P]UTP. For 5’-labeling of substrates, [γ-32P]ATP was used instead of cold ATP in the final kinasing reactions.

Directional cloning of the in vitro processing product. Adaptors for small-scale directional cloning were designed such that each contains a central BmeT110I site. The 3’ adaptor was pre-adenylated using an established protocol (Song et al. 2015). Briefly, 1 nmole of 5’-
phosphorylated 3′ adaptor was incubated with 300 U of T4 RNA Ligase (Ambion) in the presence of 25%(v/v) PEG 8000 (New England Biolabs) in a 500-μl reaction at 37°C for 2 hr. The adenylated adaptor was gel-purified on a 20% urea-polyacrylamide gel. The RNA fragments to be characterized were gel-purified and ligated to the pre-adenylated 3′ adaptor using T4 RNA Ligase 2, truncated (New England Biolabs) in the presence of 25%(v/v) PEG 8000 at 16°C overnight. The ligation reaction was separated on a 15% urea-polyacrylamide gel and the ligated RNA was gel-purified. The purified RNA was then ligated to the 5′ adaptor using T4 RNA Ligase in the presence of 25%(v/v) PEG 8000 at 22°C overnight. The RNA ligated to both adaptors was reverse transcribed using PrimeScript Reverse Transcriptase (Takara) and amplified by Phusion High-Fidelity DNA Polymerase. The PCR product was digested with BmeT110I (Takara) and treated with T4 DNA Ligase (Takara) to form concatemers, which were subsequently cloned into the TA Cloning Vector and sequenced. The sequences of adaptors and primers used for directional cloning are listed in Supplemental Table 1.

Recombinant protein purification. His-GST (from empty pHis-GST) and His-GST-T6B were purified by immobilized metal ion affinity chromatography. Briefly, 500 ml of bacterial culture was harvested, resuspended in 15 ml of ice-cold Buffer P500 (20 mM Tris-HCl at pH 7.4, 500 mM NaCl, and 2 mM βME), and sonicated. The crude lysate was cleared by centrifugation at 15,000 rpm at 4°C for 20 min and the resulting supernatant was filtered through a 0.22 μm polyethersulfone Millex-GP Syringe Filter Unit (EMD Millipore). The cleared lysate was applied to a HisTrap HP column (GE Healthcare). The column was washed with 20 ml of Buffer P500/I50 (20 mM Tris-HCl at pH 7.4, 500 mM NaCl, 2 mM βME, and 50 mM imidazole) and the immobilized protein was eluted with 5 ml of Buffer P250/I500 (20 mM Tris-HCl at pH 7.4, 250 mM NaCl, 2 mM βME, and 500 mM imidazole). The eluate was loaded into the SnakeSkin Dialysis Tubing (10 kDa cut-off; Thermo Fisher Scientific) and dialyzed against 2 L of Storage Buffer I [1× PBS, 20%(v/v) glycerol, and 1 mM DTT] at 4°C overnight. His-GST-PARN (wild-type or D28A) was batch-purified using GST-Bind Agarose Resin (Elpis Biotech). To this end, the bacterial pellet was resuspended in ice-cold Buffer T500 [20 mM Tris-HCl at pH 7.4, 500 mM KCl, 0.2 mM EDTA, and 10%(v/v) glycerol], and lysed and cleared as described above. The cleared lysate was supplemented with Triton X-100 at a final concentration of 10%(v/v) and incubated with the resin (1 ml per 500 ml culture) at 4°C for 1 hr with constant rotation.
The resin was washed with 10 ml of Buffer T500 three times and the bound protein was eluted with 5 ml of GST Elution Buffer (100 mM Tris-HCl at pH 7.4, 100 mM KCl, 1 mM DTT, and 10 mM reduced glutathione). The eluate was dialyzed against 2 L of Storage Buffer II [20 mM Tris-HCl at pH 7.4, 100 mM KCl, 0.2 mM EDTA, 20% (v/v) glycerol, and 1 mM DTT] at 4°C overnight. The concentration of purified protein was determined by Bradford assay (Protein Assay Dye Reagent Concentrate; Bio-Rad) or by densitometric analysis in parallel with serial dilutions of BSA on a SDS-polyacrylamide gel. The purified protein was distributed into single-use aliquots, snap-cooled with liquid nitrogen, and stored at -80°C.

**Quantitative RT-PCR (qRT-PCR).** To measure mRNA levels, total RNA isolated with Tri Reagent (Thermo Fisher Scientific) was treated with Recombinant DNase I (Takara) and reverse transcribed with oligo(dT)_{20} (Bioneer) and PrimeScript Reverse Transcriptase. The resulting cDNA was amplified with LightCycler 480 SYBR Green I Master (Roche Life Science). The human GAPDH mRNA was amplified in parallel as an endogenous control and the fold changes were calculated based on the comparative Ct method. Primer sequences are listed in Supplemental Table 1. To detect miRNA expression, TaqMan MicroRNA Assays (Thermo Fisher Scientific) were performed according to the manufacturer’s instructions. All qRT-PCR experiments were conducted with the LightCycler 480 Instrument II (Roche Life Science).

**miRNA targeting study.** For reporter construction, the miRNA target site of interest was inserted into the XhoI/NotI sites of psiCHECK-2 (Promega). For miRNA targeting study, HeLa S3 cells pre-treated with siRNAs for 72 hr were transfected with ~100 nM anti-miRNAs (Bioneer) (and 50 ng of empty vector or targeting reporter construct per well of a 24-well plate in the case of reporter assays) using Lipofectamine 2000 (Thermo Fisher Scientific) and incubated further for 24 hr. The cells were harvested for RNA extraction or luciferase reporter assays. The Dual-Luciferase Reporter Assay System (Promega) was used for luciferase reporter assays. The fold derepression was calculated as \((RL_{\text{reporter}}/FL_{\text{reporter}})/(RL_{\text{empty}}/FL_{\text{empty}})\), where RL and FL indicate the signals from the Renilla and firefly luminescence, respectively.
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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. In vitro DROSHA processing of pri-miR-362, Related to Figure 1. (A) In vitro DROSHA processing of human and mouse pri-miR-362. A body-labeled pri-miRNA substrate of ~300 nt in length was incubated with the Microprocessor complex immunopurified from HEK293T cells overexpressing DROSHA-FLAG and V5-DGCR8 for 30 min and the reaction products were analyzed by phosphorimaging. Pre-miR-362 liberated by the Microprocessor is indicated. The dashed line indicates discontinuous lanes from the same gel. (B) Mapping of DROSHA cleavage sites on pri-miR-362. To increase the specificity, we mapped the 5’ and the 3’ cleavage sites separately, by pairing one primer complementary to the adaptor and the other to the internal sequence of pre-miR-362. The frequency of each cleavage site is indicated, with the major cleavage sites marked in bold. Note that mouse pri-miR-362 is processed into 59 nt or 60 nt precursors to a similar extent. We annotated the 59 nt fragment as mouse pre-miR-362 because it carries a canonical 2 nt overhang.

Supplemental Figure 2. Characterization of in vitro DICER processing products for pre-miR-362, Related to Figure 1. Sanger sequencing reads for the DICER processing products are presented. The frequency of each cleavage site is indicated on the right side, with the major cleavage sites marked in bold.

Supplemental Figure 3. In vitro trimming of miR-362-5p loaded into other AGO proteins, Related to Figure 2. (A) In vitro trimming of miR-362-5p loaded into four different human AGO proteins. Trimming assays were performed essentially as in Figure 2C, except for utilizing HEK293T lysate overexpressing the indicated FLAG-AGO protein for miR-362-5p loading. (B) In vitro trimming of miR-362-5p loaded into AGO2(Y311A). (C) Western blot analysis demonstrating the comparable expression of AGO proteins used in miR-362-5p loading. The dashed line indicates discontinuous lanes from the same gel. Alpha-tubulin served as a loading control.

Supplemental Figure 4. Validation of RNAi-mediated knockdown, Related to Figure 3. (A) Knockdown of each 3’-to-5’ exoribonuclease candidate tested in Figure 3A was confirmed by qRT-PCR. Error bars represent SEM from two independent experiments. (B) Successful
depletion of PARN in Figure 3D was demonstrated by western blot analysis.

**Supplemental Figure 5. Changes in the fraction of 3′-extended miRNA reads in PARN KO cells, Related to Figure 7.** (A) Changes in the fraction of 3′-extended miRNA isoforms upon PARN ablation. For 247 miRNAs whose genome-matching counts exceed 1,000 in parental HeLa S3 cells, “Fraction +1”, “Fraction +2”, and “Fraction +3” were calculated individually in parental and PARN KO cells. The 5p and 3p miRNAs are shown in blue and turquoise, respectively. (B) Changes in the 3′-extended fraction for miR-224-5p. The miRBase sequence of miR-224-5p is 21 nt in length, but PARN trims this miRNA from 24 nt to 22 nt, rather than to 21 nt, such that the vast majority of miR-224-5p isoforms are longer than 21 nt (Figure 7C). This explains the little change in “Fraction +1” of miR-224 upon PARN deletion, despite its high sensitivity to PARN, and rationalizes extending the analysis to “Fraction +2” and “Fraction +3”.

**Supplemental Figure 6. PARN-mediated trimming in other cell lines and its possible evolutionary conservation in vertebrates, Related to Figure 7.** (A) PARN-mediated miRNA trimming occurs in other cell types. PARN was depleted in four different human cell lines and the expression of miRNAs that were efficiently trimmed by PARN in HeLa S3 cells was examined by northern blot analysis. Knockdown efficiency was confirmed by western blotting (the last two rows). The purple bars indicate the shorter miRNA isoforms plausibly generated by PARN, the fraction of which was calculated for each miRNA and is plotted on the right side. Error bars and asterisks represent SEM and statistical significance from two independent experiments, respectively (* p<0.05, *** p<0.005, one-tailed paired t-test). (B) The sequences of human miRNAs that undergo efficient 3′ trimming by PARN. For each miRNA, the reference sequence annotated by miRBase is shown in red. With the exception of miR-451, which is not expressed in HeLa S3 cells, these miRNAs were selected based on a cutoff of 20 percent point mean changes in any of three 3′-extended fractions. The nucleotides resected by PARN were deduced from the changes in length distribution of miRNA isoforms in the sequencing libraries, and are indicated by blue shading. (C) The sequences of bilaterian pre-miR-182 orthologues. Note that the remarkable sequence homology of pre-miR-182 does not extend beyond vertebrates. The 25th and 26th nucleotides of miR-182-5p, which are subjected to PARN-meditated trimming in human cells, are highly conserved among vertebrate
orthologues (blue shading). (D) The length distribution of miR-182-5p sequencing reads in selected vertebrate species. The reads from *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis*, and *Danio rerio* were obtained from miRGeneDB2.0 (http://mirgenedb.org/) with default options.

**Supplemental Figure 7. AGO protein affinity purification by peptides (AGO-APP), Related to Figure 7.** (A) Coomassie brilliant blue (CBB) staining of the recombinant proteins used in AGO-APP. (B) AGO-APP precipitates all human AGO proteins to a comparable efficiency. HEK293T cytoplasmic lysate containing the indicated FLAG-AGO protein was incubated with GST-T6B or GST immobilized on glutathione-agarose beads. After washing, co-purifying proteins were eluted and subjected to western blot analysis. Alpha-tubulin served as a loading control for input lysates and a negative control for AGO-APP. (C) AGO-APP is capable of isolating endogenous miRISC. Whole cell lysate from untreated HeLa S3 cells was subjected to AGO-APP and co-purifying RNAs were extracted. Enrichment of miRNAs in the AGO-APP precipitate was examined by TaqMan MicroRNA assays. U6 snRNA served as a negative control for AGO-APP.

**Supplemental Figure 8. The impact of PARN-mediated 3′ trimming on miRNA targeting.** (A) Northern blot analysis of total RNAs from manipulated HeLa S3 cells. HeLa S3 cells pre-treated with control siRNA (siGFP) or siPARN were transfected with anti-miR-182-5p or anti-miR-1-3p. We used anti-miR-1-3p as a negative control because the cognate miRNA, miR-1-3p, is not expressed in HeLa S3 cells. The fraction of the shorter miR-182-5p isoforms in anti-miR-1-3p-treated cells was calculated as in Supplemental Fig. 6A and plotted on the right side. The error bar represents SEM from three biologically independent experiments, and the asterisks indicate statistical significance of the decrease in the fraction of the shorter isoforms calculated by one-tailed paired *t*-test (** *p*<0.005) (B) Derepression of *FRS2* mRNA expression upon blocking miR-182-5p activity. The relative abundance of the *FRS2* mRNA in each sample was measured by qRT-PCR. (C) One of the four miR-182-5p target sites present in the *FRS2* mRNA 3′ UTR, which was used for luciferase reporter construction. (D) Derepression of miR-182-5p reporter gene expression upon anti-miR-182-5p treatment. In (B) and (D), error bars represent SEM from three biologically independent experiments, and asterisks indicate statistical significance of the increase in the relative *FRS2* mRNA level or
reporter gene activity calculated by one-tailed paired $t$-test (**) $p<0.01$, *** $p<0.005$). In (B) and (D), there were no significant differences in the extent of derepression between mock- and PARN-depleted cells (n.s., not significant, two-tailed paired $t$-test).

**Supplemental Figure 9. Changes in the modification frequency and the mean tail length in PARN KO cells, Related to Figure 8.** (A) Changes in the length of miRNA tails upon PARN deletion. (B) Tailing frequency of individual miRNAs in parental and PARN KO cells. The fraction of prefix-matching reads was calculated for the 267 miRNAs with total read counts exceeding 1,000 in parental cells. The 5p and 3p miRNAs are colored blue and turquoise, respectively, which also applies to all the other scatter plots in this figure. (C) Mean changes in the tailing frequency upon PARN ablation were plotted over miRNA abundance in parental cells. (D) The frequency of mono-adenylation or mono-uridylation of individual miRNAs in parental and PARN KO cells ($n = 267$). (E) Northern blot analysis of miR-17/20a, miR-18a, and miR-19a/b. For simultaneous detection of paralogous miRNAs with nearly identical sequences (miR-17-5p and miR-20a, and miR-19a-3p and miR-19b-3p), we used degenerated oligonucleotides as probes. See Supplemental Table 1. (F) The mean length of adenosine or uridine tails in parental and PARN KO cells. For each type of tail, miRNAs with mono-nucleotide-added read counts exceeding 100 in parental cells were used for the analysis ($n = 198$ for adenosine tails and $n = 190$ for uridine tails). (G) Boxplots depicting changes in the mean tail length of individual miRNAs upon PARN deletion.

**Supplemental Figure 10.** Effects of CUGBP1 on miR-362-5p trimming in HEK293T cells. CUGBP1 was (A) overexpressed or (B) depleted in HEK293T cells and the maturation of ectopically expressed miR-362-5p was monitored by northern blotting. Overexpression and depletion of CUGBP1 were confirmed by western blotting and qRT-PCR, respectively. Note that the blot presented in (B) is the same as that presented in Fig. 3A. The dashed lines indicate discontinuous lanes from the same gel.
Supplemental Figure 1

A

In vitro DROSHA processing

hsa-mmu- Pri-miR-362
- + - + : DROSHA/DGCR8

Pre-miR-362

B

Human pri-miR-362

Mouse pri-miR-362
Supplemental Figure 3

**A**

*In vitro* trimming of hsa-miR-362-5p

| AGO1 | AGO2 | AGO3 | AGO4 |
|------|------|------|------|
| 0    | 0.5  | 1    | 2    |

**B**

*In vitro* trimming of hsa-miR-362-5p

| AGO2 | AGO2(Y311A) |
|------|-------------|
| 0    | 0.5         |

**C**

Western blot

Mock AGO1 AGO2 AGO3 AGO4 Mock AGO2(Y311A)

μ-globin α-tubulin
Supplemental Figure 4

A

qRT-PCR

Relative transcript level

EXOSC10, DIS3, DIS3L, DIS3L2, PARN, ER11

: Knockdown in HEK293T

B

Western blot

Transfection in HeLa S3

siGFP, siPARN

PARN, α-tubulin

1, 2
Supplemental Figure 5

A

Fraction +1

Fraction +2

Fraction +3

B

Two predominant reads in parental HeLa S3 cells

miRBase annotation

Genome-matching reads

3' end annotated by miRBase

miR-224-5p

Fraction +1 Fraction +2 Fraction +3

miR-224-5p

PARN KO#1 PARN KO#2

PARN KO#1 PARN KO#2

PARN KO#1 PARN KO#2

Parental Parental Parental

Parental Parental Parental

5′...GCTTTCAAGTCACTAGTGGTTCCGTTTAGTA...3′

3′...CGAAAGTTCAGTGATCACCAAGGCAAATCAT...5′

5′-CAAGUCACUAGUGGUUCCGUUUAGU -3′

5′-CAAGUCACUAGUGGUUCCGUUUAG -3′

5′-CAAGUCACUAGUGGUUCCGUUUA -3′

5′-CAAGUCACUAGUGGUUCCGUU -3′

5′-CAAGUCACUAGUGGUUCCGU -3′

5′-CAAGUCACUAGUGGUUCCG -3′

Fraction +1

Fraction +2

Fraction +3

miR-224-5p

miR-224-5p

miR-224-5p

miRBase annotation

Genome-matching reads

Two predominant reads in parental HeLa S3 cells

miRBase annotation

miR-224-5p

Fraction +1

Fraction +2

Fraction +3

miR-224-5p

PARN KO#1 PARN KO#2

PARN KO#1 PARN KO#2

PARN KO#1 PARN KO#2

Parental Parental Parental

Parental Parental Parental

5′...GCTTTCAAGTCACTAGTGGTTCCGTTTAGTA...3′

3′...CGAAAGTTCAGTGATCACCAAGGCAAATCAT...5′

5′-CAAGUCACUAGUGGUUCCGUUUAGU -3′

5′-CAAGUCACUAGUGGUUCCGUUUAG -3′

5′-CAAGUCACUAGUGGUUCCGUUUA -3′

5′-CAAGUCACUAGUGGUUCCGUU -3′

5′-CAAGUCACUAGUGGUUCCGU -3′

5′-CAAGUCACUAGUGGUUCCG -3′

Fraction +1

Fraction +2

Fraction +3

miR-224-5p

miR-224-5p

miR-224-5p

miRBase annotation

Genome-matching reads

Two predominant reads in parental HeLa S3 cells

miRBase annotation

miR-224-5p

Fraction +1

Fraction +2

Fraction +3

miR-224-5p

PARN KO#1 PARN KO#2

PARN KO#1 PARN KO#2

PARN KO#1 PARN KO#2

Parental Parental Parental

Parental Parental Parental

5′...GCTTTCAAGTCACTAGTGGTTCCGTTTAGTA...3′

3′...CGAAAGTTCAGTGATCACCAAGGCAAATCAT...5′

5′-CAAGUCACUAGUGGUUCCGUUUAGU -3′

5′-CAAGUCACUAGUGGUUCCGUUUAG -3′

5′-CAAGUCACUAGUGGUUCCGUUUA -3′

5′-CAAGUCACUAGUGGUUCCGUU -3′

5′-CAAGUCACUAGUGGUUCCGU -3′

5′-CAAGUCACUAGUGGUUCCG -3′

Fraction +1

Fraction +2

Fraction +3

miR-224-5p

miR-224-5p

miR-224-5p

miRBase annotation

Genome-matching reads

Two predominant reads in parental HeLa S3 cells

miRBase annotation

miR-224-5p

Fraction +1

Fraction +2

Fraction +3

miR-224-5p

PARN KO#1 PARN KO#2

PARN KO#1 PARN KO#2

PARN KO#1 PARN KO#2

Parental Parental Parental

Parental Parental Parental

5′...GCTTTCAAGTCACTAGTGGTTCCGTTTAGTA...3′

3′...CGAAAGTTCAGTGATCACCAAGGCAAATCAT...5′

5′-CAAGUCACUAGUGGUUCCGUUUAGU -3′

5′-CAAGUCACUAGUGGUUCCGUUUAG -3′

5′-CAAGUCACUAGUGGUUCCGUUUA -3′

5′-CAAGUCACUAGUGGUUCCGUU -3′

5′-CAAGUCACUAGUGGUUCCGU -3′

5′-CAAGUCACUAGUGGUUCCG -3′

Change in fraction

(PARN KO − Parental)
Supplemental Figure 6

A. PARN manipulation in other cells

| Cell Line | Mock | PARN KD | Rescue |
|-----------|------|---------|--------|
| A549      |      |         |        |
| Huh7      |      |         |        |
| MCF7      |      |         |        |
| HEK293T   |      |         |        |

B. Human miRNAs efficiently trimmed by PARN

| miRNA     | Sequence                  |
|-----------|---------------------------|
| hsa-ac-pre-miR-451a | AAACCGUUACCAUACUGGUUAAGUAGG |
| hsa-miR-425-5p  | AUGCUGAACAGCUCCCGGGAGUAGG |
| hsa-miR-362-5p  | UUUGCCAUCGACUGGGUGGAGGUGGC |
| hsa-miR-361-3p  | CAGUGCCAUUGUGUGGGUGGGAAGAC |
| hsa-miR-182-5p  | UAGUGCAAUAUGGUUCCGUUUAG |
| hsa-miR-301a-3p | AUGCACCUGGGCAAGGAUUCUGA |
| hsa-miR-500a-3p | CAGUGCAAUAGUAUUGUCAAAGCAU |
| hsa-miR-224-5p  | AACAUUCAACGCUGUCGGUGAGUUU |
| hsa-miR-454-3p  | CAAGUCACUAGUGGUUCCGUUUAG |
| hsa-miR-342-3p  | UAGUGCAAUAUUGCUUAUAGGGUUU |
| hsa-miR-2110-5p | UCUCACACAGAAAUCGCACCCGUCA |
| hsa-miR-330-3p  | UUGGGGAAACGGCCGCUGAGUGAG |

C. Primary sequences of pre-miR-182 orthologues

| Orthologue | Sequence                  |
|------------|---------------------------|
| hsa-pre-miR-182 | UUUGCCAACGCGAUGUGUGGUUCUAGA--CUUGCCAACUA |
| mmu-pre-miR-182 | UUUGCCAACGCGAUGUGUGGUUCUAGA--CUUGCCAACUA |
| gga-pre-miR-182 | UUUGCCAACGCGAUGUGUGGUUCUAGA--CUUGCCAACUA |
| xtr-pre-miR-182 | UUUGCCAACGCGAUGUGUGGUUCUAGA--CUUGCCAACUA |
| dre-pre-miR-182 | UUUGCCAACGCGAUGUGUGGUUCUAGA--CUUGCCAACUA |
| dme-pre-miR-263b | UUUGCCAACGCGAUGUGUGGUUCUAGA--CUUGCCAACUA |
| cel-pre-miR-790 | UUUGCCAACGCGAUGUGUGGUUCUAGA--CUUGCCAACUA |

D. Length distribution of miR-182-5p sequencing reads

| Species     | Mock | PARN KD | Rescue |
|-------------|------|---------|--------|
| Parental HeLa S3 |      |         |        |
| PARN K012    |      |         |        |
| M. musculus  |      |         |        |
| G. gallus    |      |         |        |
| X. tropicalis|      |         |        |
| D. rerio     |      |         |        |

Fraction of reads
Supplemental Figure 7

A. CBB staining

B. Western blot

| Input (10%) | GST pulldown | GST pulldown | GST pulldown |
|------------|--------------|--------------|--------------|
| AGO1 | AGO2 | AGO3 | AGO4 | AGO1 | AGO2 | AGO3 | AGO4 | : Bait |
| : HEK293T cytoplasmic lysate |
| FLAG-AGO |
| α-tubulin |

C. AGO-APP from HeLa S3 lysate followed by TaqMan miRNA assay

- miR-21-5p
- miR-16-5p
- let-7-5p
- miR-122-5p
- U6 snRNA

Relative RNA level
Supplemental Figure 8

A. Northern blot

- - + + : siPARN
- - + + : anti-miR-182-5p

miR-182-5p
Pre-miR-182
U6 snRNA

Densitometry

Fraction of shorter isoforms in mock-treated cells

B. qRT-PCR

Targeting by endogenous miR-182-5p

qRT-PCR

relative FRS2 mRNA level

** ***

n.s.

C. Reporter assay

miR-182-5p

5′-UUUGGCAUUUGGUACGAAACUCACACUCUGG-3′

3′-...AUGUUAAACCGUUACCAUCAGGGUGACAACCUGUUA...-5′

FRS2 3′ UTR (4th target site)

Insert into Renilla luciferase (Rluc) 3′ UTR

D. Reporter assay

Targeting by endogenous miR-182-5p

Rluc/Fluc luminescence

** ***

n.s.
Supplemental Figure 10

A

Northern blot
Transfection in HEK293T

Mock
FLAG-CUGBP1

(-) - (+) : hsa-pri-miR-362
Pre-miR-362

miR-362-5p

5S rRNA

(NT)

70

40

29

19

60

50

Western blot

M 1 2 3

β-actin

Mock

FLAG-CUGBP1

Northern blot
Transfection in HEK293T

siGFP
siCUGBP1

(NT)

70

40

29

19

60

50

5S rRNA

Fraction of shorter isoform (24 nt)

Mock : 0.412
FLAG-CUGBP1 : 0.424

B

Northern blot
Transfection in HEK293T

M 1 2 3 4 M 1 2 3 4

β-actin

Mock

FLAG-CUGBP1

Northern blot
Transfection in HEK293T

siGFP
siCUGBP1

(NT)

70

40

29

19

60

50

5S rRNA

qRT-PCR

Relative CUGBP1 mRNA level

siGFP : 0.469
siCUGBP1 : 0.433

Fraction of shorter isoform (24 nt)

Mock : 0.412
FLAG-CUGBP1 : 0.424

siGFP : 0.469
siCUGBP1 : 0.433

1.00
0.31