Supplemental Information

PARN mediates 3′-end trimming of Argonaute2-cleaved precursor microRNAs

Mayuko Yoda, Daniel Cifuentes, Natsuko Izumi, Yuriko Sakaguchi, Tsutomu Suzuki, Antonio J. Giraldez and Yukihide Tomari

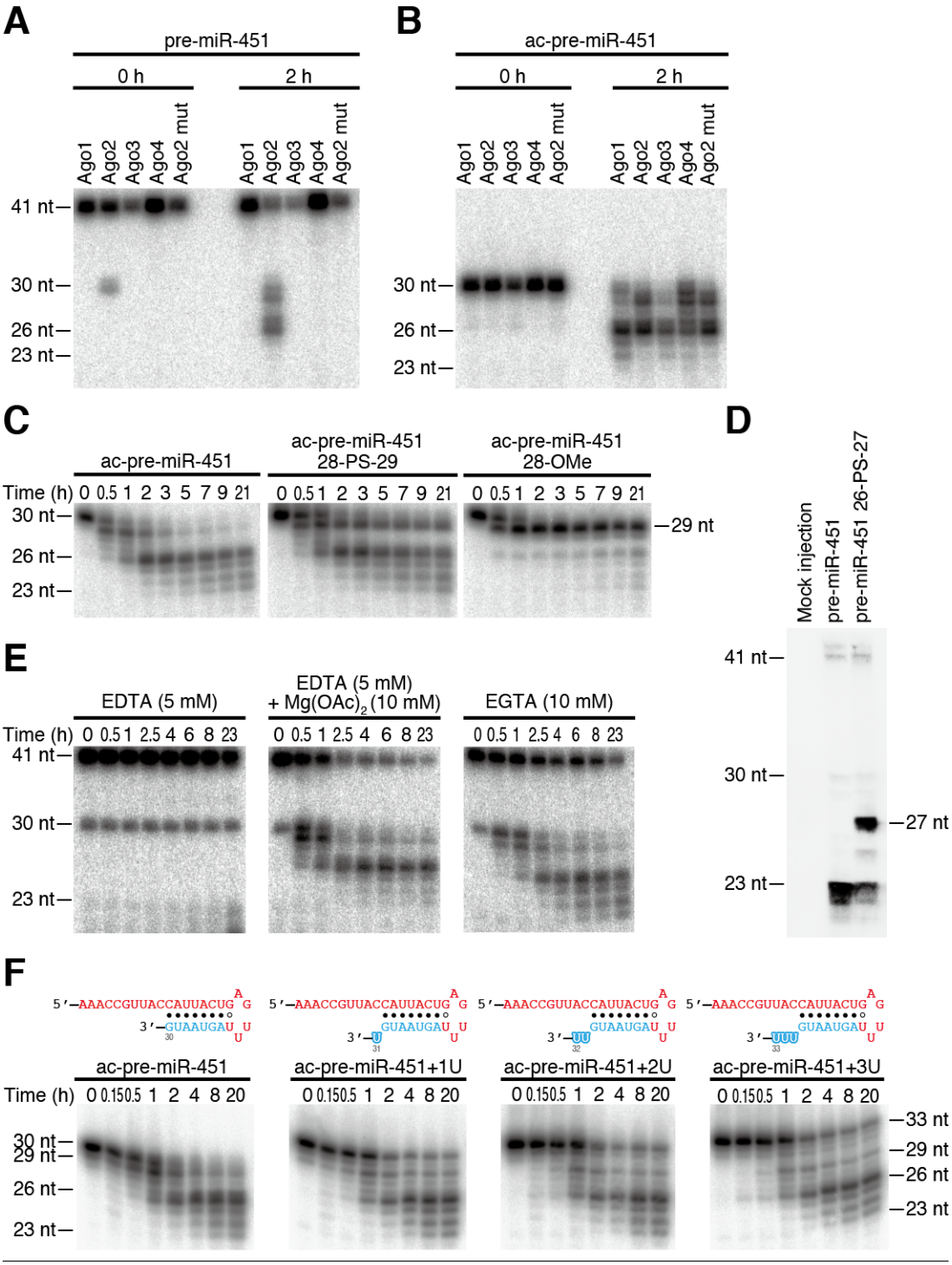

Figure S1. Biochemical characterization of the trimming reaction, Related to Figure 1.

(A) Cleavage of the pre-miR-451 3′ arm is required for trimming in vitro. Pre-miR-451 was loaded into FLAG-tagged Ago1–4 or the catalytic mutant of Ago2 on beads and

trimmed in K562 lysate. Mature miR-451 was produced only with the catalytically active wild-type Ago2.

(B) Pre-nicked ac-pre-miR-451 can be matured regardless of the catalytic activity of Ago. Ac-pre-miR-451 was loaded into FLAG-tagged Ago1–4 or the catalytic mutant of Ago2 on beads and trimmed in K562 lysate. Mature miR-451 was produced even with catalytically inactive Ago proteins.

(C and D) The trimming enzyme is an exoribonuclease. Introduction of a phsphorotioate linkage or a 2′-*O*-methyl group specifically inhibited the trimming reaction at the corresponding positions in vitro (C) and in zebrafish embryos (D).

(E) The trimming enzyme is Mg^{2+}-dependent. Trimming was inhibited by EDTA and rescued by an excess amount of Mg^{2+}.

(F) Oligouridylation does not affect trimming. Ac-pre-miR-451 and its derivatives with additional 1–3 uridines at the 3′ end were assayed for trimming in K562 lysate.

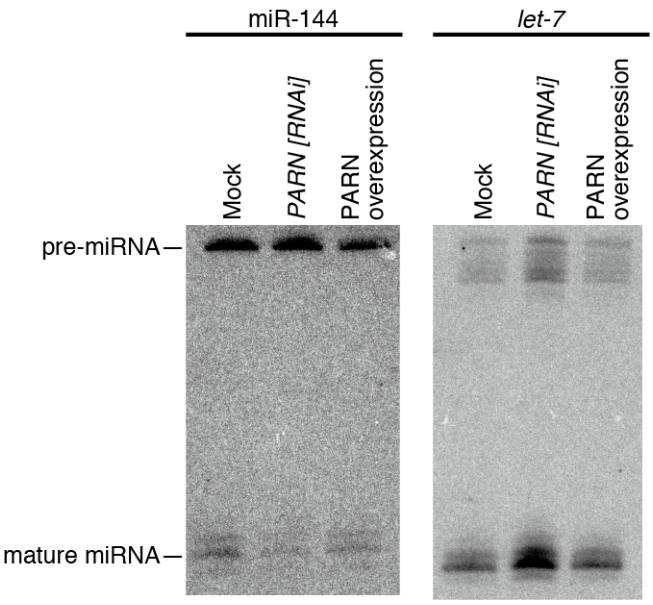

Figure S2. Knockdown or overexpression of PARN does not affect the lengths of miR-144 and endogenous *let-7,* Related to Figure 2.

Note that PARN knockdown markedly inhibited the cell growth and reduced the expression level of miR-144/miR-451, while having little impact on endogenous *let-7*. Importantly, unlike miR-451 (Figure 2D), the lengths (not the abundance) of miR-144 and *let-7* were not affected by knockdown and overexpression of PARN, highlighting the specific role of PARN in miR-451 maturation.

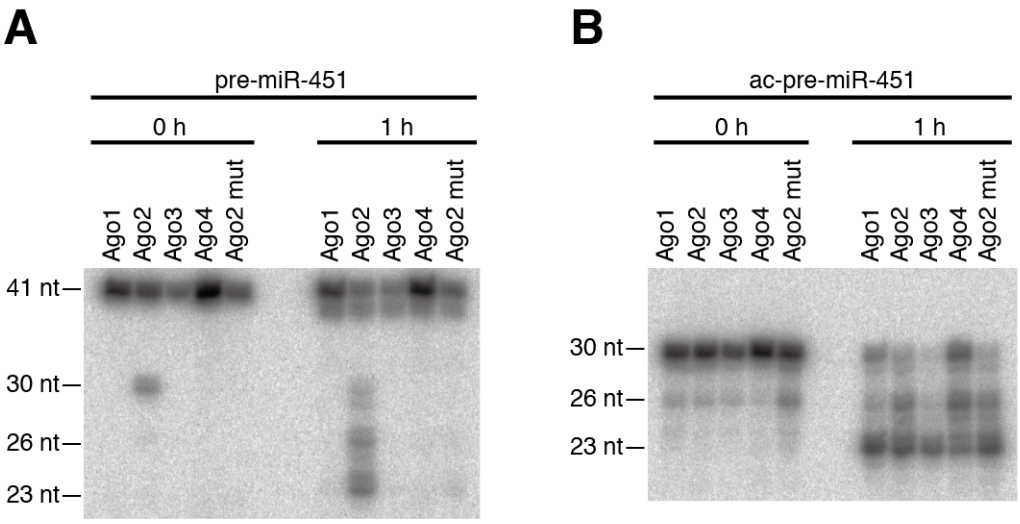

Figure S3. Trimming by recombinant PARN, Related to Figure 3.

(A) Pre-miR-451 was loaded into FLAG-tagged Ago1–4 or the catalytic mutant of Ago2 on beads and trimmed by recombinant PARN. Mature miR-451 was produced only with the catalytically active wild-type Ago2.

(B) Ac-pre-miR-451 was loaded into FLAG-tagged Ago1–4 or the catalytic mutant of Ago2 on beads and trimmed by recombinant PARN. Mature miR-451 was produced even with catalytically inactive Ago proteins.

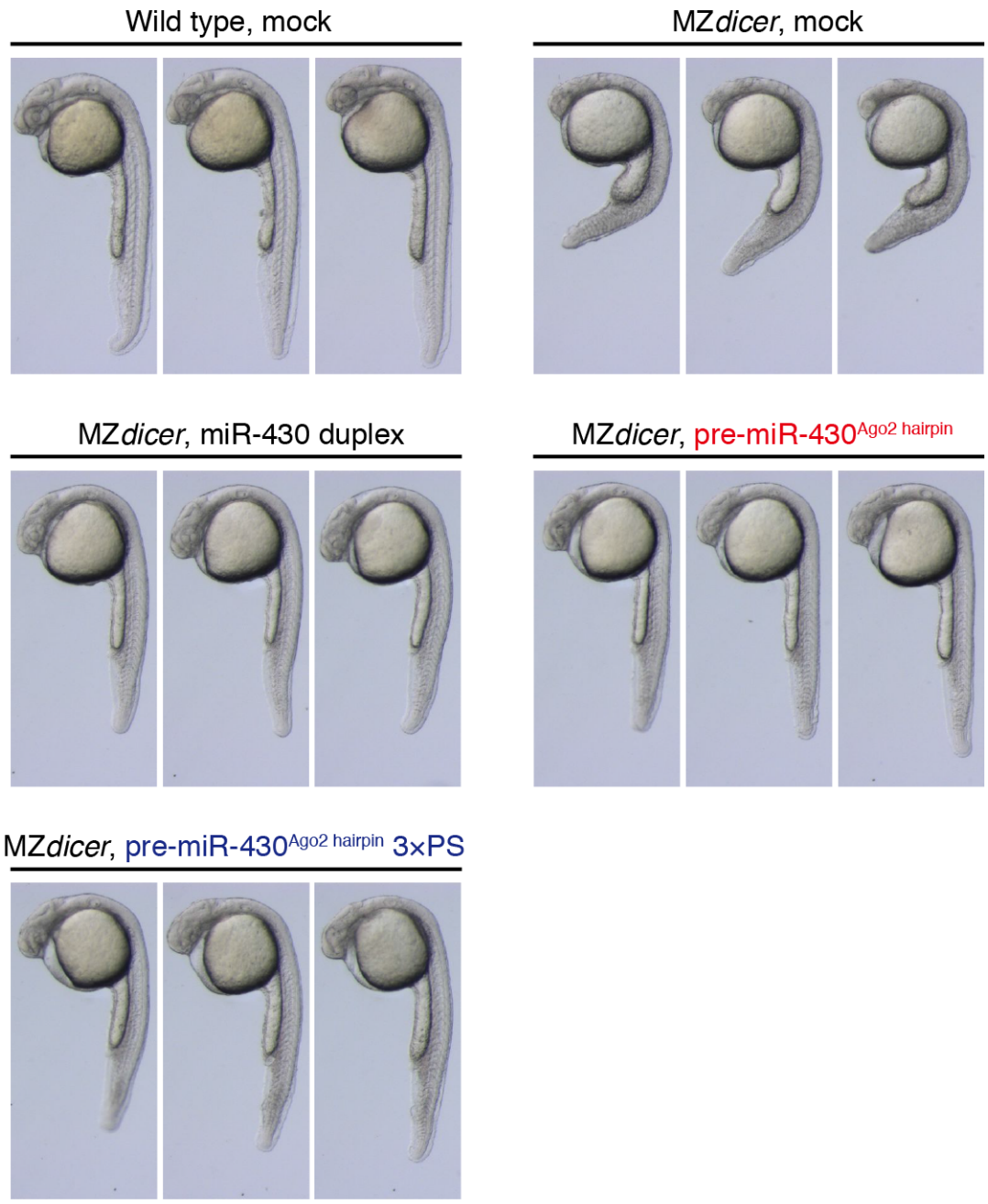

Figure S4. Trimming is not essential to rescue the MZ*dicer* phenotype, Related to Figure 5.

Injection of miR-430 duplex, pre-miR-430$^{Ago2\ hairpin}$ or pre-miR-430$^{Ago2\ hairpin}$ 3×PS rescued the morphological defects of MZ*dicer* mutant embryos. Three representative embryos for each condition are shown.

Table S1. Summary of RNA sequencing, Related to Figure 6.

Genotype	Injection	Total number of reads	Reads aligning by TopHat	Reads aligning to genes
Wild type	–	37,116,664	31,990,244	26,223,728
MZ*dicer*	–	21,187,083	18,204,781	14,840,805
MZ*dicer*	miR-430 duplex	35,702,972	30,873,614	25,256,403
MZ*dicer*	pre-miR-430$^{\text{Ago2 hairpin}}$	34,707,968	29,534,030	24,074,005
MZ*dicer*	pre-miR-430$^{\text{Ago2 hairpin}}$ 3×PS	35,019,312	30,101,667	24,576,382