Expanded View Figures

Figure EV1. Loss of Zuc and Gasz impacts the cellular localization of Armi and Piwi in OSCs.

A Western blotting using anti-Gasz monoclonal antibody shows that the Gasz signal disappeared upon Gasz RNAi treatment in OSCs. Anti-Gasz monoclonal antibody was produced in this study. β-Tubulin (β-Tub) was detected as a loading control.

B Western blotting shows that the levels of Armi and Piwi in the cytoplasmic fraction after mitochondrial isolation (cyto) were little impacted by Zuc depletion, while the level of Armi in the mitochondrial fraction (mito) increased.

C Larger immunostaining views of the cell images shown in Fig 1C and D. The cells appeared in the main figures are boxed with white dotted line. In "Zuc KD" panel, the upper cell appeared in Fig 1C, while the lower one appeared in Fig 1D. Armi behaved similarly in the cells other than the one shown in Fig 1C and D. The scale bar represents 5 μm.

D Upper: Localization of Piwi (green) in OSCs upon Zuc and Gasz depletion. Yb bodies are shown in red. Lower: Larger immunostaining views of the cell images shown in upper panels. The cells appeared in the upper panels are boxed with white dotted line. Piwi was mislocalized in the cytosol upon the loss of Zuc and/or Gasz. Some fraction of Piwi was detected at Yb bodies as has previously been reported [24]. The scale bar represents 5 μm. DAPI (blue) shows the nuclei.

E Left: Localization of Armi (green) in OSCs upon Gasz depletion. Mitochondria are shown in red. Right: Larger immunostaining views of the cell images shown on the left. The scale bar represents 5 μm. DAPI (blue) shows the nuclei.

F Piwi localizes to Yb bodies after Armi has localized there. Upon Piwi–piRISC formation by Zuc on the outer membrane of mitochondria, Armi goes back to Yb bodies, while Piwi–piRISC is translocated to the nucleus for silencing. The details and requirements of inter-organelle translocation of Armi and Piwi–pre-piRISC remain unknown (red dotted line with an arrowhead).

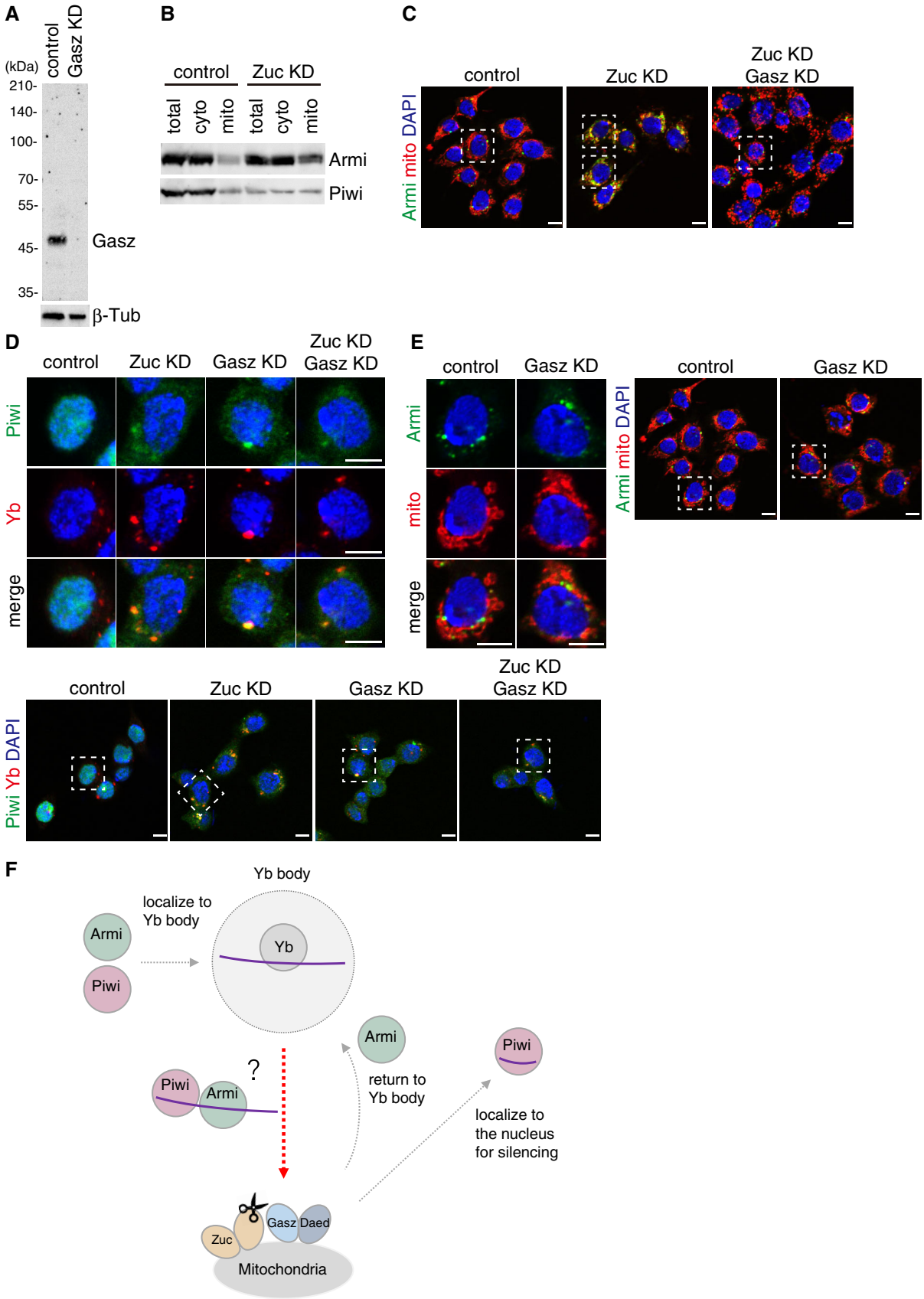

Figure EV1.

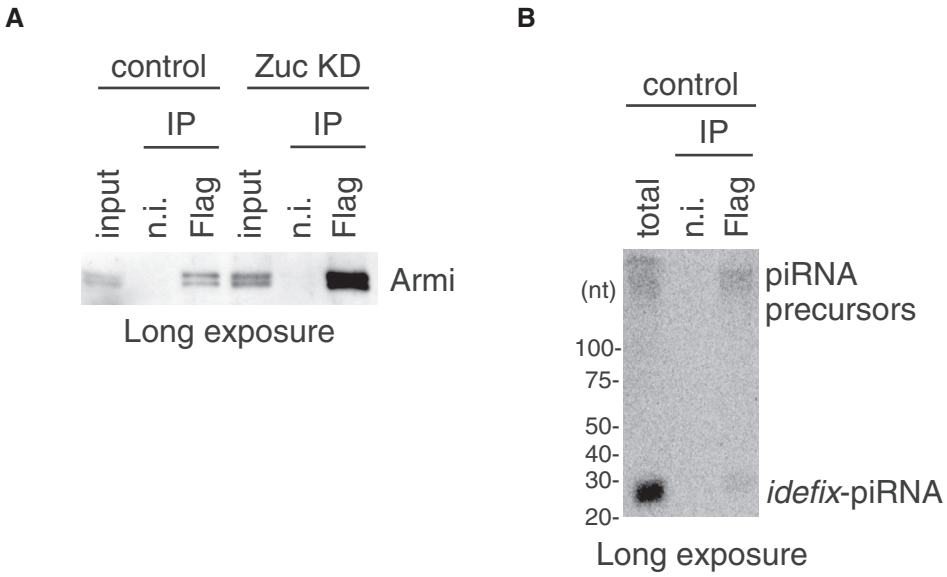

Figure EV2. Gasz binds Armi–Piwi–pre-piRNA on the outer membrane of mitochondria.

A This Western blot is identical to the blot in Fig 2B (Armi) but with a longer exposure time.
B This northern blot is identical to the blot in Fig 2C (left half) but with a longer exposure time.

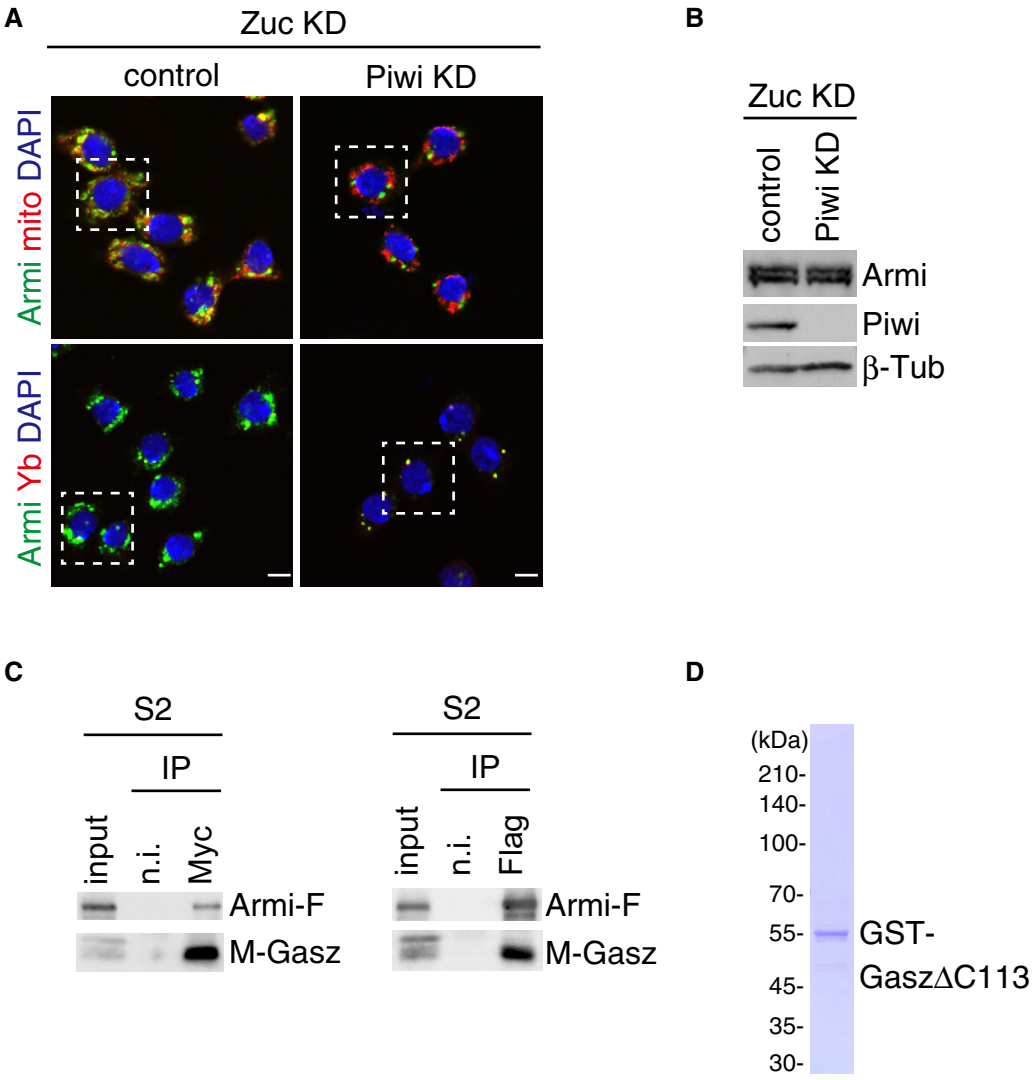

Figure EV3. Departure of Armi from Yb bodies depends on Piwi.

A Larger immunostaining views of the cell images shown in Fig 3A and B. The cells appeared in the main figures are boxed with white dotted line. Armi behaved similarly in the cells other than the one shown in Fig 3A and B. The scale bar represents 5 μm.

B Piwi depletion hardly affected the level of Armi in Zuc-depleted OSCs. β-Tubulin (β-Tub) was detected as a loading control.

C Myc-Gasz (M-Gasz) and Armi-Flag (Armi-F) interacted with each other in Schneider 2 (S2) cells.

D Purified recombinant GST-Gasz (ΔC113 lacking Leu349-Ser461 containing the TM region at the C-terminus) used in the experiment shown in Fig 3E. The protein was stained with CBB.

Figure EV4. Departure of Armi from Yb bodies requires piRNA precursor loading onto Piwi.

A Upper: Another set of cell images of the one shown in Fig 4B. The scale bar represents 5 μm. DAPI (blue) shows the nuclei. Lower: The expression levels of Flag-Piwi (F-Piwi) WT and PAZ/MID mutants (PAZmt/MIDmt) in Zuc/Piwi-depleted OSCs (Zuc KD + Piwi KD). β-Tubulin (β-Tub) was detected as a loading control.

B Upper: Armi localization to Yb bodies observed in Zuc/Piwi-depleted OSCs (Zuc KD + Piwi KD) (Fig 3A and B) was rescued by ectopic expression of Flag-Piwi WT and PAZmt but not of MIDmt. "Rescued" means that Armi is now localized to mitochondria as in Zuc-depleted OSCs (Fig 1C). The scale bar represents 5 μm. DAPI (blue) shows the nuclei. Lower: Another set of cell images of the one shown on the left. The scale bar represents 5 μm. DAPI (blue) shows the nuclei.

C Upper: The cellular localization of Flag-Piwi (F-Piwi) WT and PAZ/MID mutants (PAZmt/MIDmt) (yellow), Yb (red), and Armi (green) in Piwi-depleted OSCs (Piwi KD). Middle: Another set of cell images of the one shown on the left. The scale bar represents 5 μm. DAPI (blue) shows the nuclei. Lower: The expression levels of Flag-Piwi (F-Piwi) WT and PAZ/MID mutants (PAZmt/MIDmt) in Piwi-depleted OSCs (Piwi KD). β-Tubulin (β-Tub) was detected as a loading control.

D Armi has three functions. [A] Armi's Yb-body localization is required for Piwi localization at Yb bodies. [B] Armi senses and binds Piwi–pre-piRISC at Yb bodies. This action of Armi is required for inter-organelle translocation of the complex from Yb bodies to mitochondria. [C] Armi relaxes piRNA precursors for Zuc cleavage on the mitochondria.

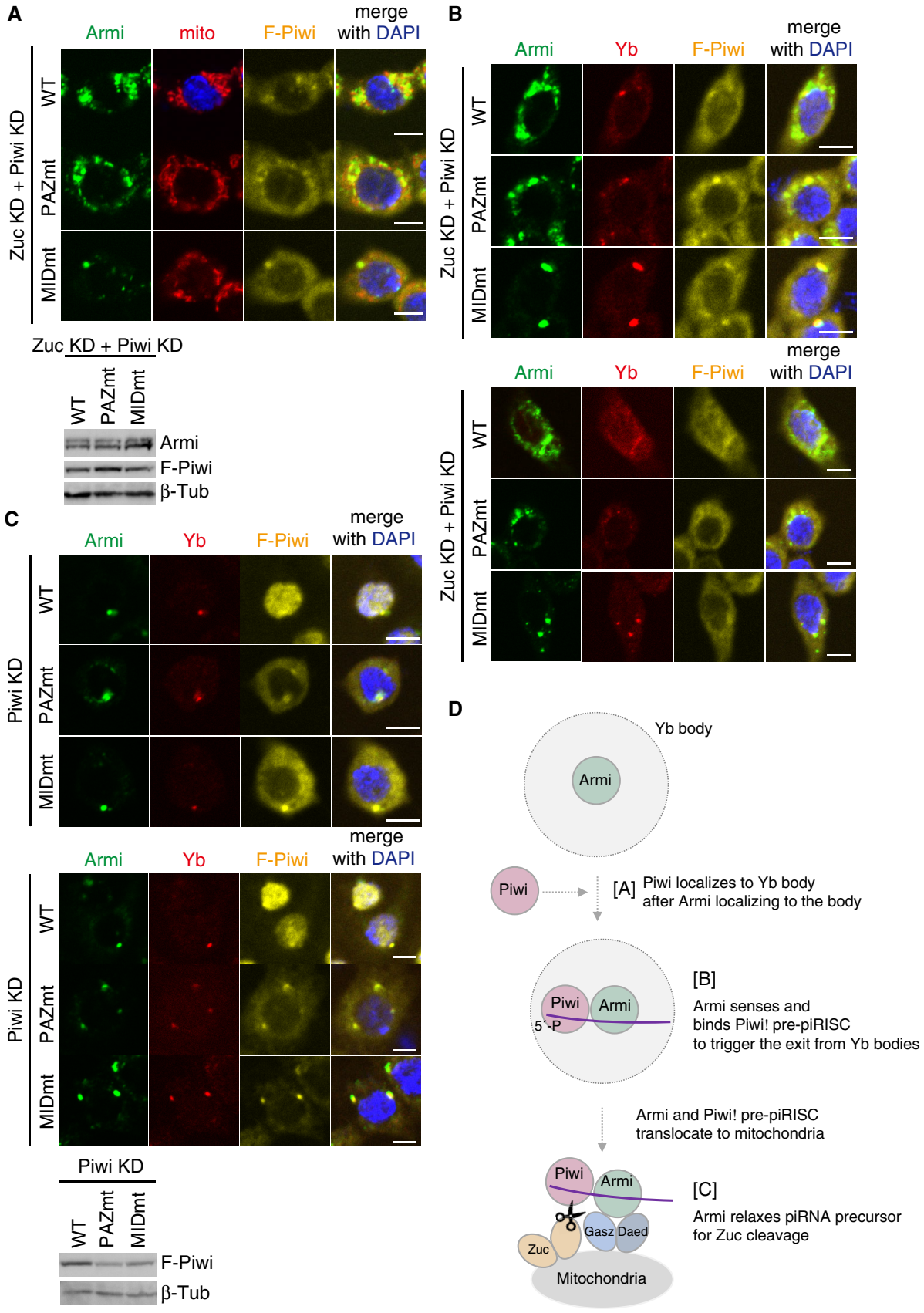

Figure EV4.

Figure EV5. The behaviors of Armi N756A mutant and ΔN34 mutant.

A Left: Purified recombinant Armi-Flag (Armi-F) WT and mutants (ΔN34 and N756A) used in the experiment. Right: RNA-binding activity of Armi-Flag WT, ΔN34, and N756A. Here, 16-nt single-stranded RNA was 5′-end-labeled and incubated with Armi-Flag.

B Top: Northern blotting shows that siRNA-resistant WT Armi-Flag (Armi-F), but not ΔN34 and N756A mutants, rescued the defects in *idefix*-piRNA production caused by endogenous Armi depletion. U6 snRNA was used as a loading control. Bottom: The expression levels of Armi-Flag (Armi-F) WT, ΔN34, and N756A mutants. β-Tubulin was used as a loading control.

C Left: Another set of cell images of the one shown in Fig 5B. The scale bar represents 5 μm. DAPI (blue) shows the nuclei. Right: The expression levels of Armi-Flag (Armi-F) WT and N756A mutant in Zuc/Armi-depleted OSCs (Zuc KD + Armi KD). β-Tubulin (β-Tub) was detected as a loading control.

D Left: B. Armi-Flag (Armi-F) WT and the N756A mutant were expressed in OSCs where endogenous Armi and Zuc had been depleted by RNAi (Zuc KD + Armi KD). Armi-F WT (green) localized onto mitochondria, whereas Armi-F N756A mutant (green) localized to Yb bodies (red). The scale bar represents 5 μm. DAPI (blue) shows the nuclei. Right: Another set of cell images of the one shown on the left. The scale bar represents 5 μm. DAPI (blue) shows the nuclei.

E Left: Armi-Flag (Armi-F) WT and ΔN34/N756A mutants (green) localized at Yb bodies (red) in Armi-depleted OSCs (Armi KD). Right: Another set of cell images of the one shown on the left. The scale bar represents 5 μm. DAPI (blue) shows the nuclei.

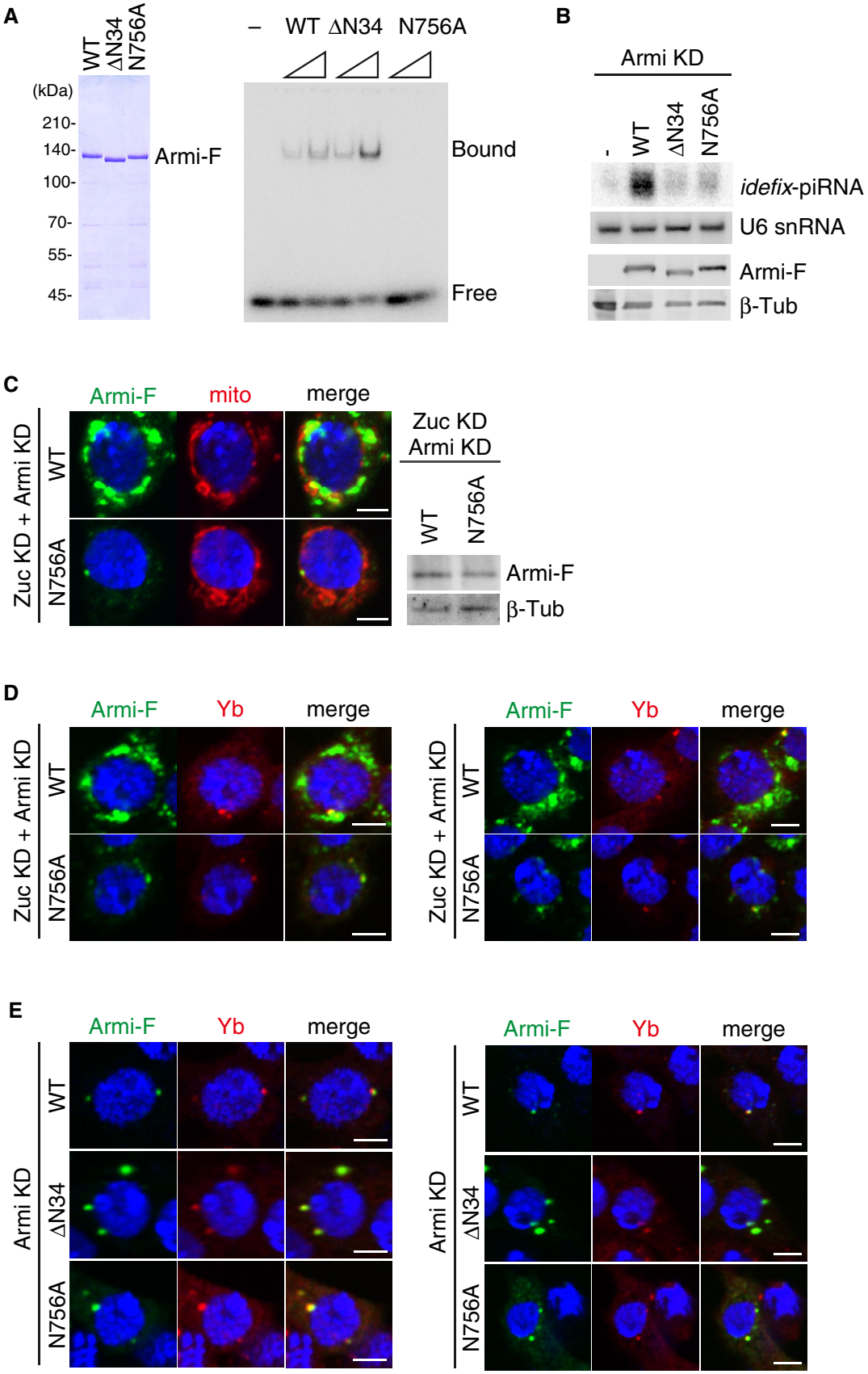

Figure EV5.

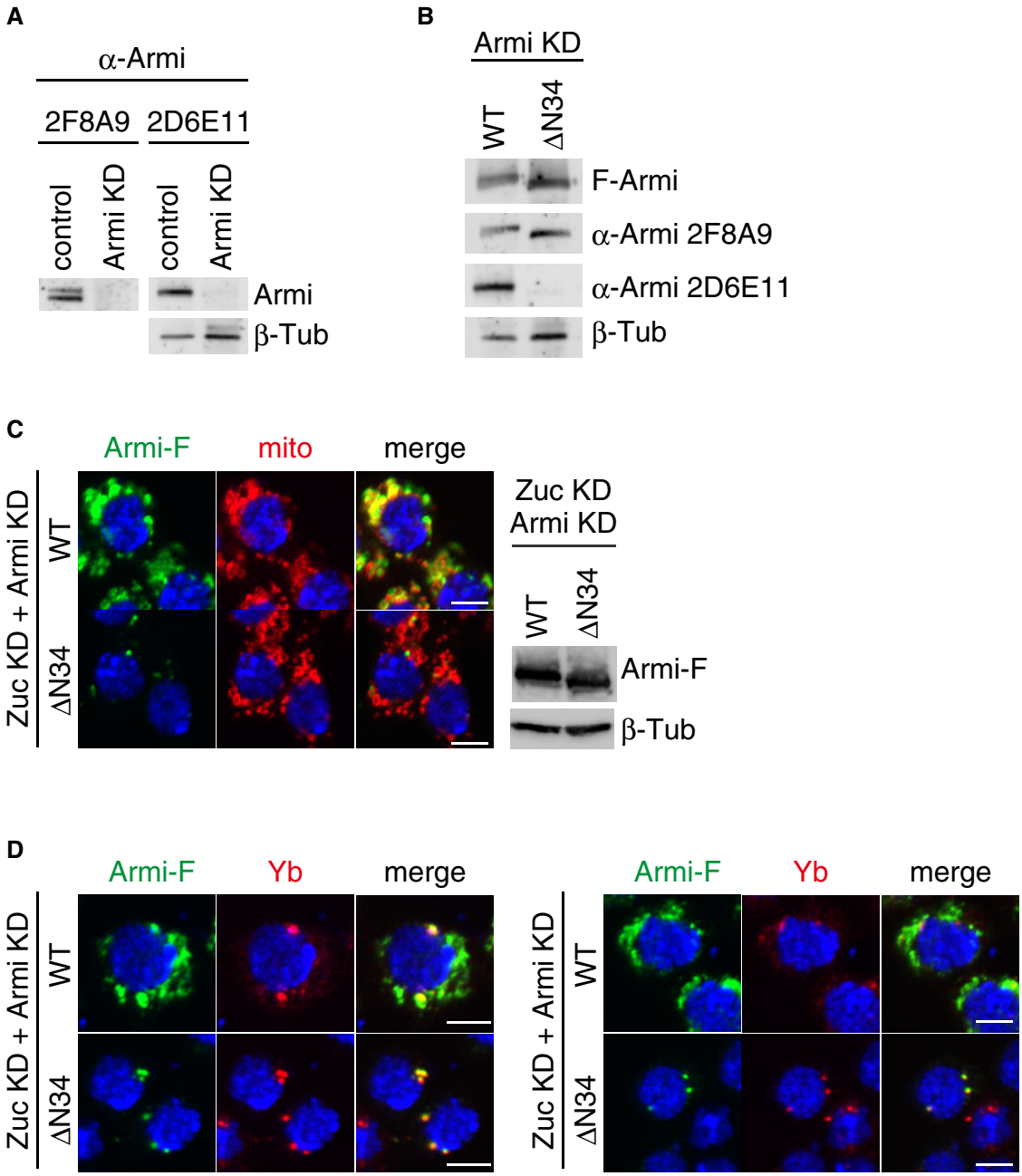

Figure EV6. The N-terminal end of Armi plays an important role in the departure of Piwi–pre-piRISC from Yb bodies.

A Anti-Armi antibody 2F8A9 recognized both Armi bands on Western blots, while 2D6E11 recognized solely the upper band. The signals of 2F8A9 were stripped away, and then, the same membrane was reprobed with 2D6E11. β-Tubulin (β-Tub) was detected as a loading control.

B Western blotting showed that 2F8A9 anti-Armi antibody recognized both Armi-Flag (Armi-F) WT and ΔN34 mutant, while 2D6E11 recognized only WT. β-Tubulin (β-Tub) was detected as a loading control.

C Left: Another set of cell images of the one shown in Fig 6B. The scale bar represents 5 μm. Right: The expression levels of Armi-Flag (Armi-F) WT and ΔN34 mutants in Zuc/Armi-depleted OSCs (Zuc KD + Armi KD). β-Tubulin (β-Tub) was detected as a loading control.

D Left: Armi-Flag (Armi-F) WT and the ΔN34 mutant were expressed in OSCs where endogenous Armi and Zuc had been depleted by RNAi (Zuc KD + Armi KD). Armi-F WT (green) localized onto mitochondria, whereas Armi-F ΔN34 mutant (green) localized to Yb bodies (red). The scale bar represents 5 μm. DAPI (blue) shows the nuclei. Right: Another set of cell images of the one shown on the left. The scale bar represents 5 μm. DAPI (blue) shows the nuclei.