Huang et al., http://www.jcb.org/cgi/content/full/jcb.201401002/DC1

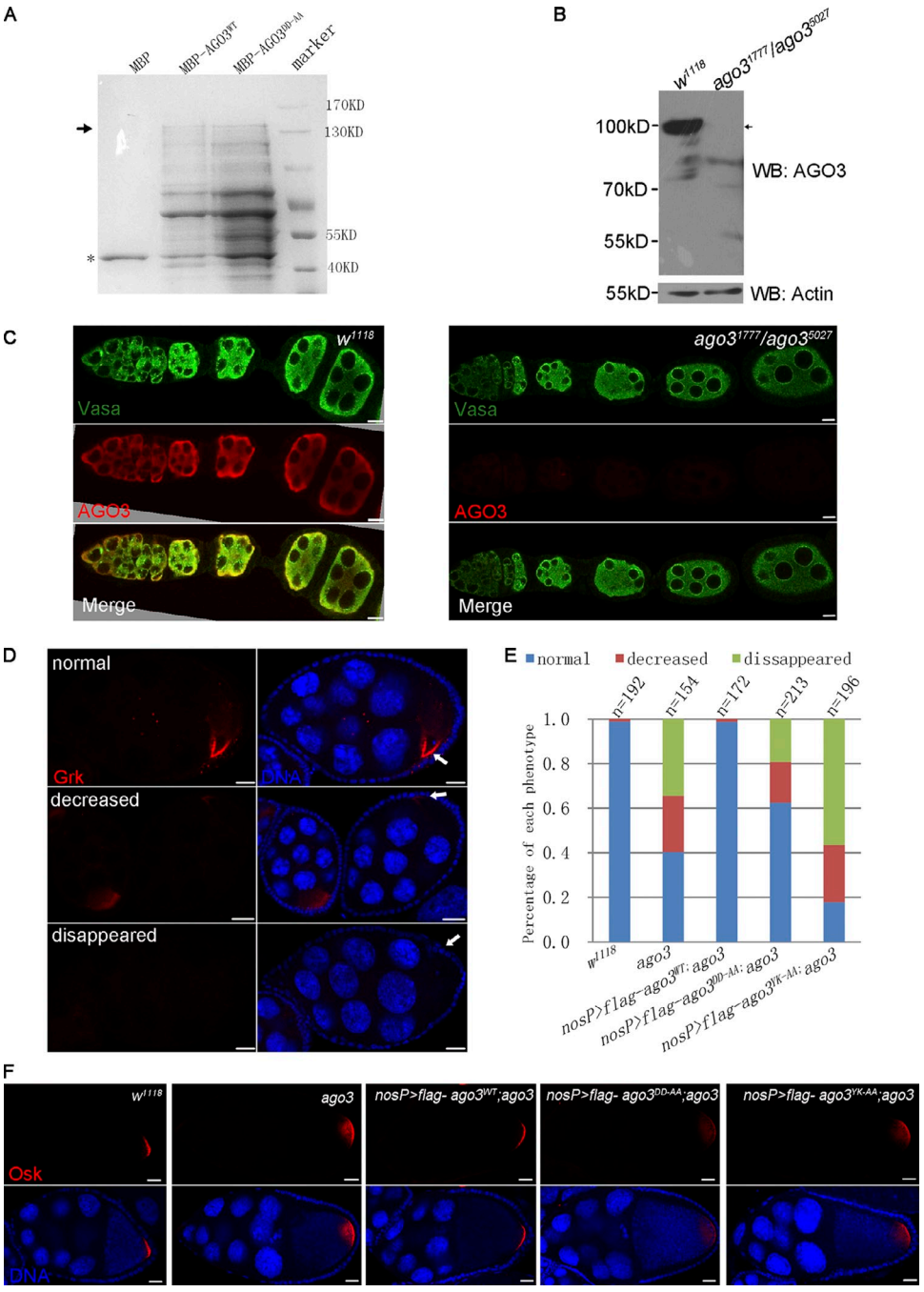

Figure S1. **AGO3 controls the expression of Grk and Osk.** (A) Coomassie blue staining of PAGE showed the expression levels of purified recombinant proteins, MBP (asterisk), full-length MBP-AGO3^{WT}, and MBP-AGO3^{DD-AA} (arrow), which were used for Slicer activity assay in Fig. 1 B. (B) Western blot using anti-AGO3 antibody showed AGO3 expression in w^{1118} and $ago3^{1777}/ago3^{5027}$ ovaries. AGO3 proteins corresponded to ~100-kD bands. (C) w^{1118} and $ago3^{1777}/ago3^{5027}$ ovaries were stained with anti-AGO3 antibody (red) and anti-Vasa antibody (green). (D and E) Ovaries were stained with anti-Grk antibody (red) and Hoechst (blue) and showed a range of expression patterns of Grk (D): (1) a normal level of Grk staining was present around the membrane overlying the oocyte nucleus that was normal in wild-type oocytes; (2) a decreased level of Grk protein that was weaker than in wild-type oocytes; and (3) no detectable Grk staining in oocyte. E shows the statistical analysis of Grk expression patterns in ovaries from w^{1118}, *ago3* mutant, *uasp-flag-ago3*^{WT}*; nosP-gal4:vp16, ago3*, *uasp-flag-ago3*$^{DD-AA}$*; nosP-gal4:vp16, ago3*, and *uasp-flag-ago*$^{YK-AA}$*; nosP-gal4:vp16, ago3*. The x axis shows genotypes of tested flies, whereas the y axis shows the percentage of ovarioles containing egg chambers with normal, decreased, and no Grk expression in different genotypes, the sample size (*n*) for each sample was indicated. Loss of *ago3* led to reduced expression of Grk proteins in oocyte, whereas germ cell–specific expression of AGO^{WT} in *ago3* mutant ovaries was sufficient to restore the proper expression pattern of Grk as seen in wild-type ovaries, but expression of either AGO3^{DD-AA} or AGO3^{YK-AA} was not. (F) Ovaries collected from w^{1118}, *ago3* mutant, *uasp-flag-ago3*^{WT}*; nosP-gal4:vp16, ago3*, *uasp-flag-ago3*$^{DD-AA}$*; nosP-gal4:vp16, ago3*, and *uasp-flag-ago*$^{YK-AA}$*; nosP-gal4:vp16, ago3* were stained with anti-Osk antibody and Hoechst. Bars, 10 µm. AGO^{WT} expression in *ago3* mutant ovaries could rescue the diffused localization of Oskar in the posterior polar, whereas either AGO3^{DD-AA} or AGO3^{YK-AA} did not.

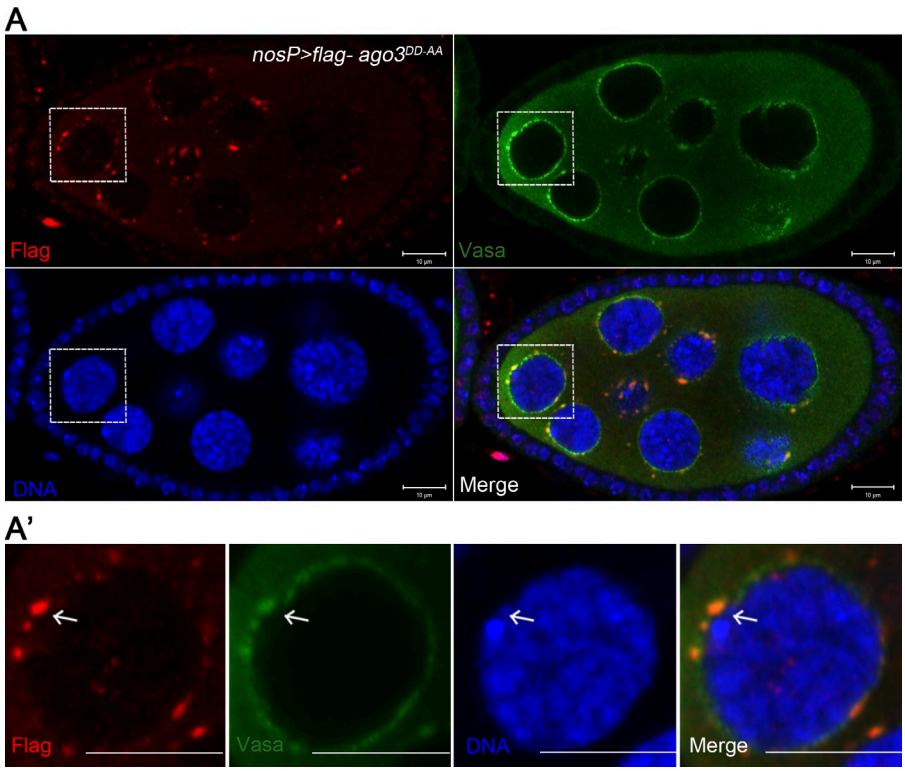

Figure S2. **Slicer mutant AGO3 was colocalized with Vasa in nuage.** (A and A′) Ovaries from *uasp-flag-ago3$^{DD\text{-}AA}$; nosP-gal4:vp16* females were stained for Flag (red), Vasa (green), and Hoechst (blue). A′ shows the enlarged image boxed in A. Bars, 10 µm.

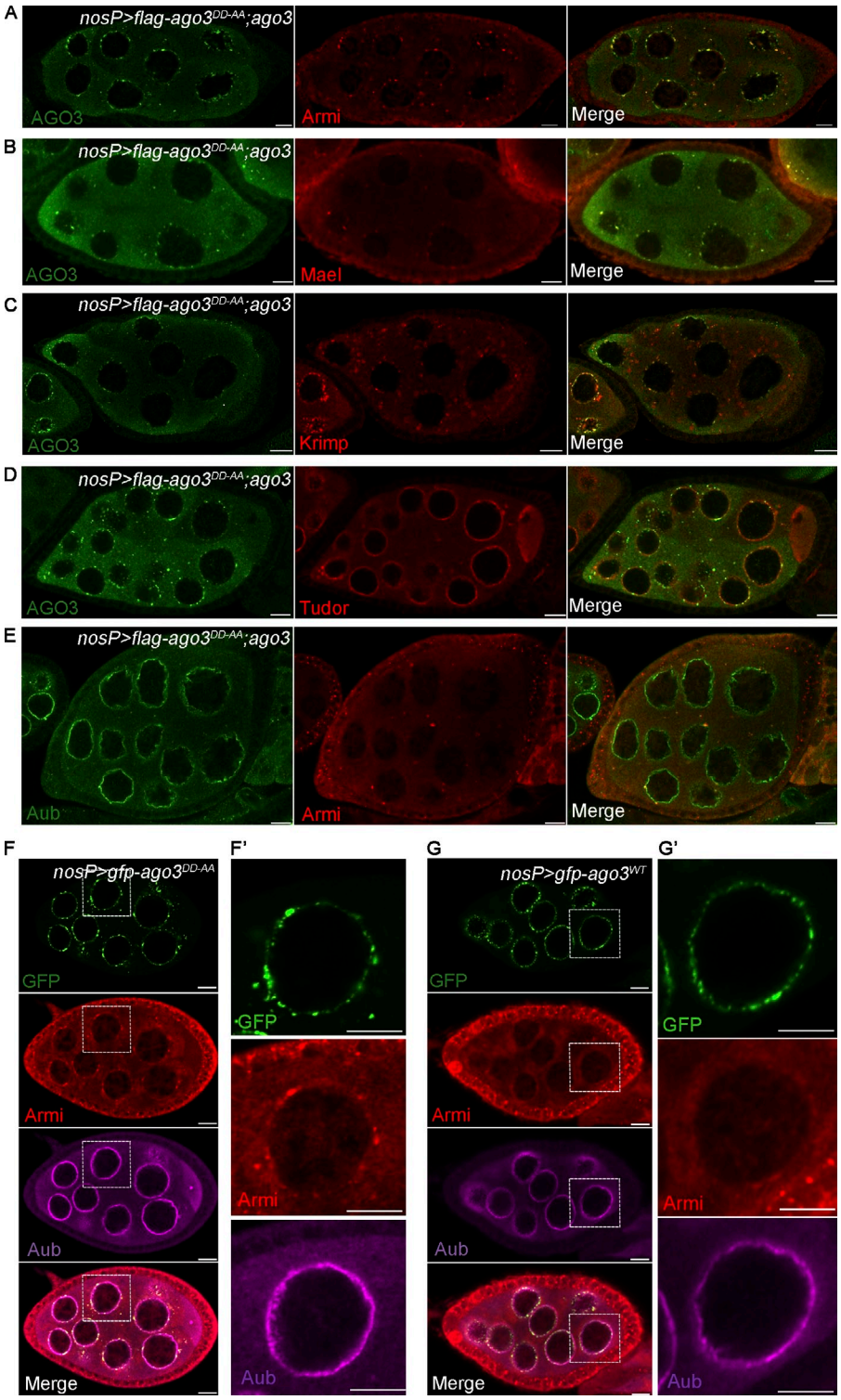

Figure S3. **Armi associates with AGO3$^{DD\text{-}AA}$ in nuage.** (A–E) Ovaries from *uasp-flag-ago3$^{DD\text{-}AA}$; nosP-gal4:vp16, ago3* females were stained for Armi and some nuage components. A, AGO3 (green) and Armi (red); B, AGO3 (green) and Mael (red); C, AGO3 (green) and Krimp (red); D, AGO3 (green) and Tudor (red); E, Aub (green) and Armi (red). (F–G′) Ovaries expressing GFP-AGO3$^{DD\text{-}AA}$ (F and F′) or GFP-AGO3^{WT} (G and G′) were stained for GFP (green), Armi (red), and Aub (pink). F′ and G′ shows the enlarged image boxed in F and G, respectively. Bars, 10 μm.

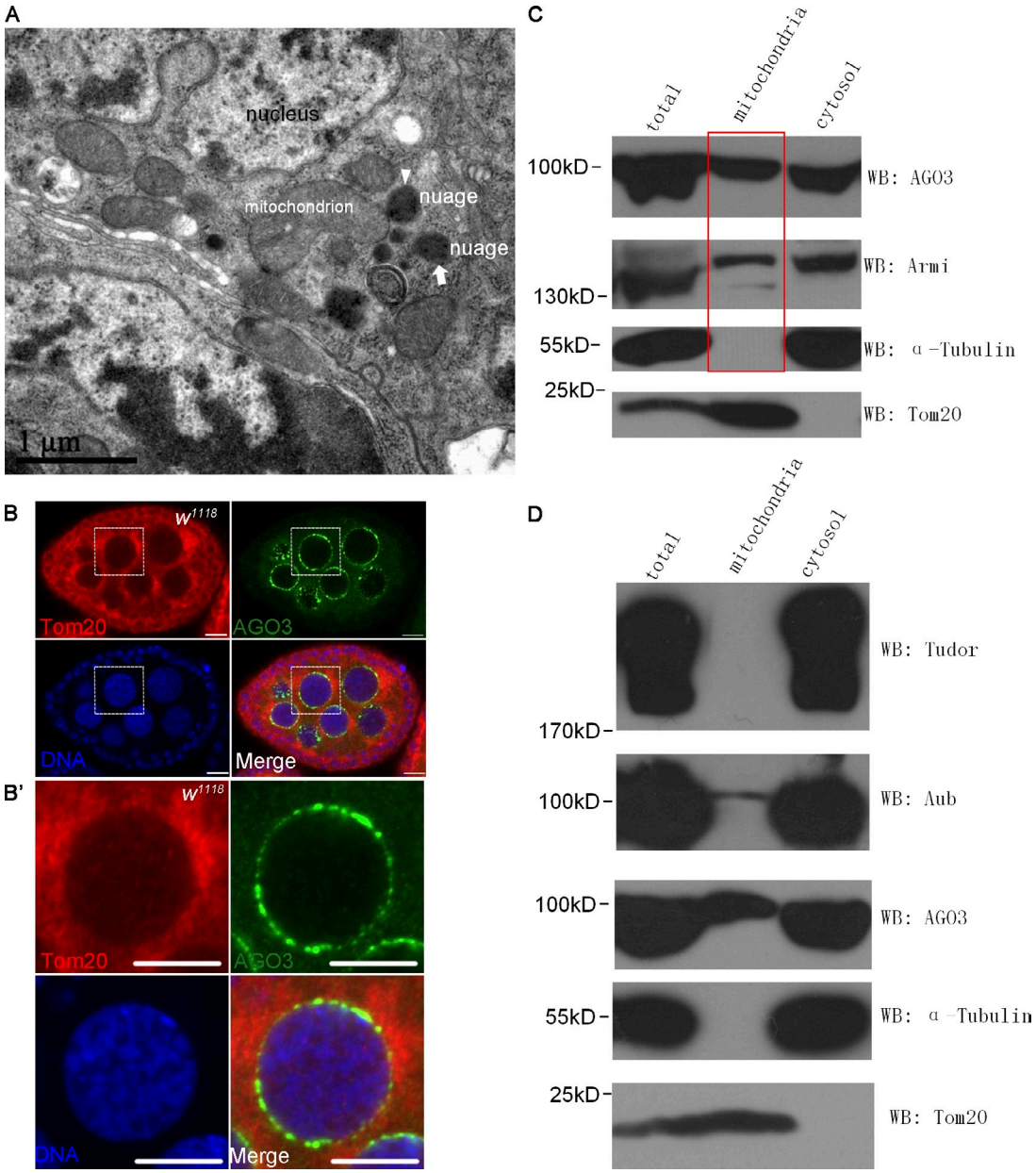

Figure S4. **AGO3 is localized at mitochondria.** (A) The electron microscope image of wild-type nurse cells showed the nuage and mitochondria structures. Some nuages were adjacent to mitochondria (arrow), whereas some were directly associated with mitochondria (arrowhead). Bar, 1 μm. (B and B′) w^{1118} ovary was stained with anti-Tom20 (red) and anti-AGO3 (green). B′ shows the enlarged images of the boxed part in B. Bars, 10 μm. (C) Western blot assays were performed to measure expression levels of AGO3, Armi, Tom20, and α-tubulin in the indicated fractions of w^{1118} ovaries. (D) Western blot assays were performed to measure expression levels of Tudor, Aub, AGO3, Tom20, and α-tubulin in the indicated fractions of w^{1118} ovaries.

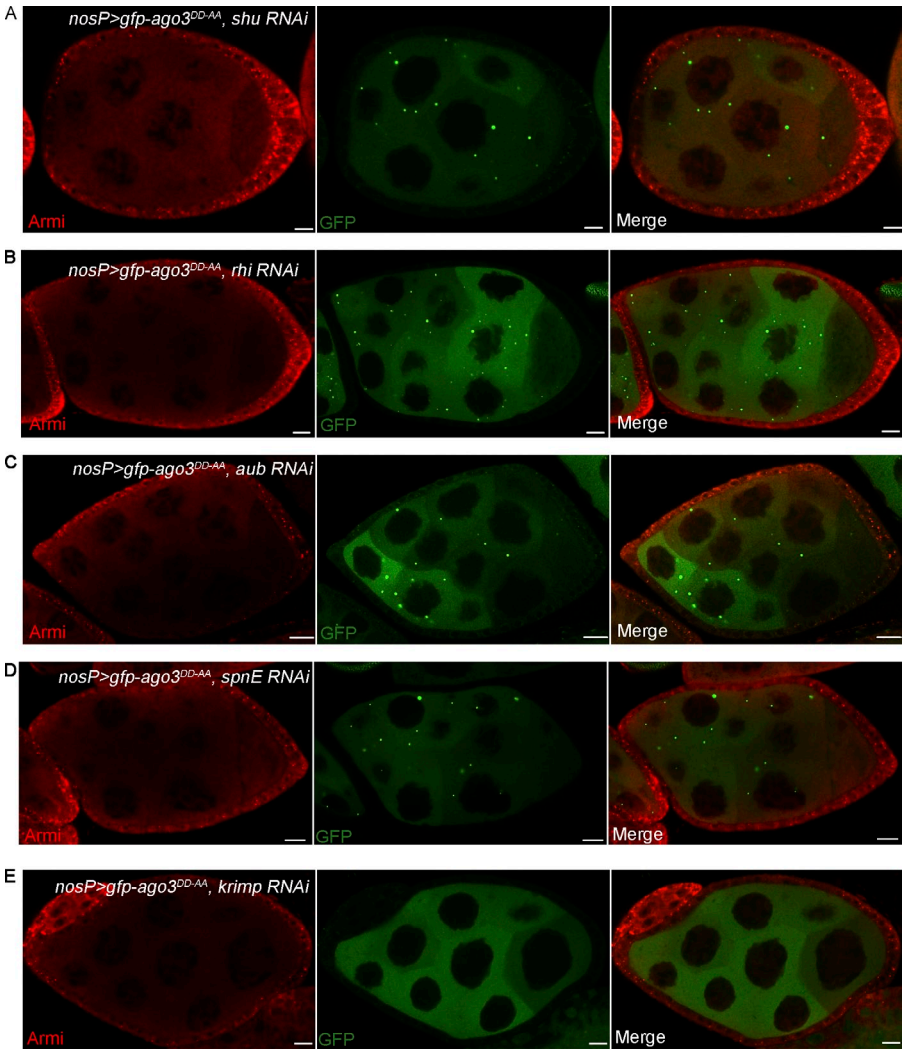

Figure S5. **Armi and AGO3 association in nuage was regulated by piRNA genes.** (A–E) Ovaries expressing GFP-AGO3$^{DD\text{-}AA}$ in the background of *shu* (A), *rhi* (B), *aub* (C), *spnE* (D), and *krimp* (E) knockdown driven by *nosP-gal4:vp16* were stained for Armi (red) and GFP (green). Bars, 10 µm.