Expanded View Figures

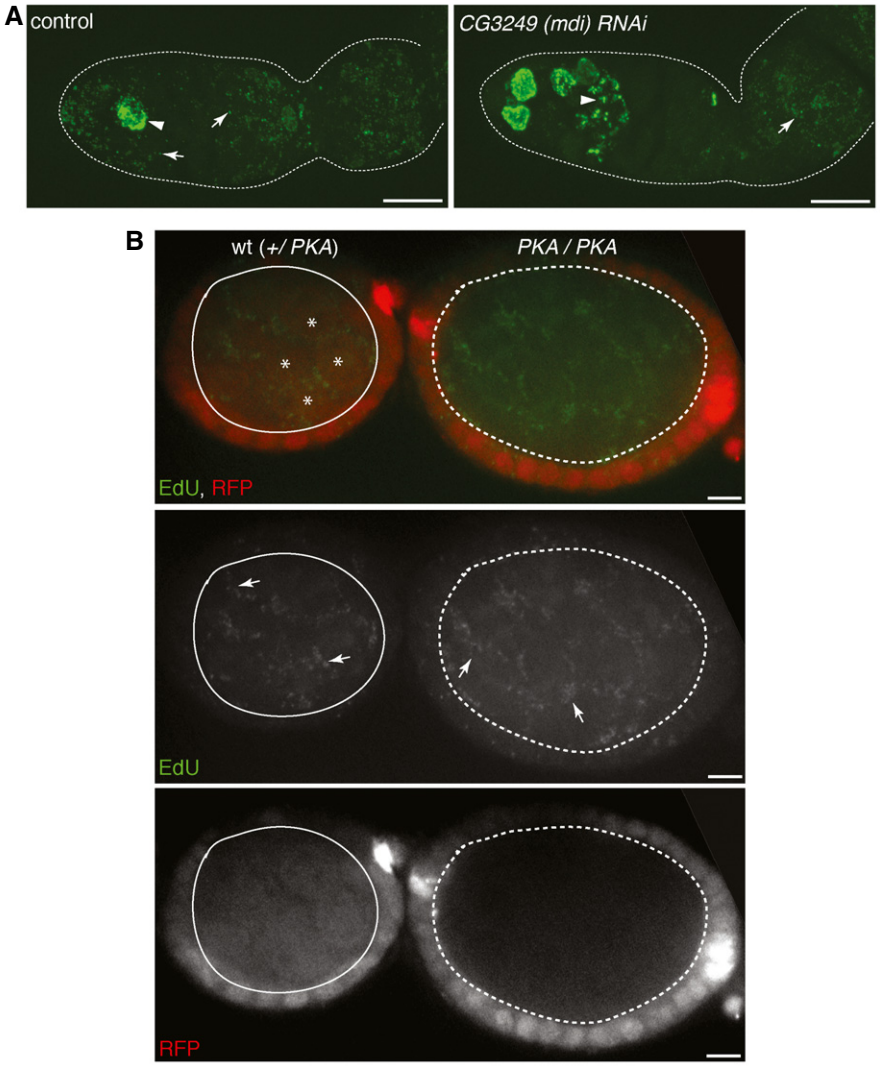

Figure EV1. mtDNA replication in *mdi* RNAi and *PKA* mutant ovaries.

A DNA replication illustrated by EdU incorporation in ovaries of control and *mdi* RNAi flies. Arrows point to mitochondria and arrowheads point to nuclei. Genotypes: *UAS-dicer*; *nos-gal4* (control); *UAS-dicer*; *nos-gal4*; *UAS-CG3249^{IR}* [*CG3249 (mdi)* RNAi]. mtDNA replication is inhibited in late germarium stage in the ovariole expressing *mdi* RNAi. Scale bars, 10 μm.

B mtDNA replication in wt and *PKA* mutant clones illustrated by EdU incorporation. Arrows point to mtDNA replication. wt clones (solid circle) are labeled with a nuclear-localized RFP reporter (*). *pka* null mutant clones that lack RFP signal (dashed circle) display normal mtDNA replication (arrows). Scale bars, 10 μm.

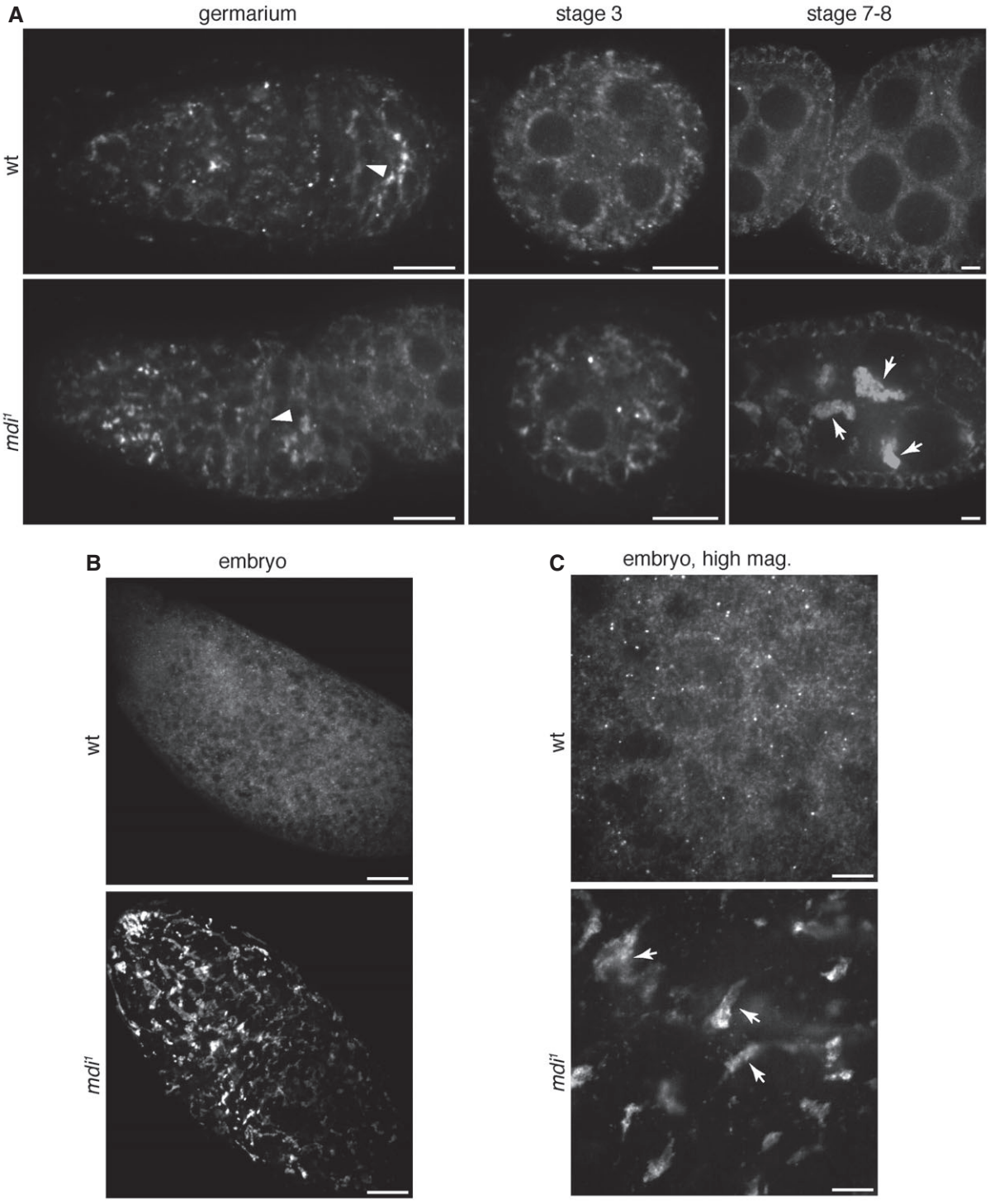

Figure EV2. Mitochondria are clumped together in the *mdi*^{1} ovary and their eggs.

A Representative images of wt and *mdi*^{1} ovarioles stained for ATP-S, illustrating mitochondrial morphology. Note that mitochondria progressively clump together in mid- to late-stage egg chambers of *mdi*^{1} flies. Scale bars, 10 μm.

B Representative images wt and *mdi*^{1} eggs stained for ATP-S, illustrating mitochondrial morphology. Scale bars, 100 μm.

C High-mag images of wt and *mdi*^{1} eggs stained for ATP-S. Scale bars, 20 μm. Note the clumping mitochondria in eggs produced by *mdi*^{1} flies.

Data information: Arrowheads point to the mitochondria that normally associate with fusome in wt and *mdi*^{1} ovarioles. Arrows point to clumped mitochondria in *mdi*^{1} ovarioles and eggs.

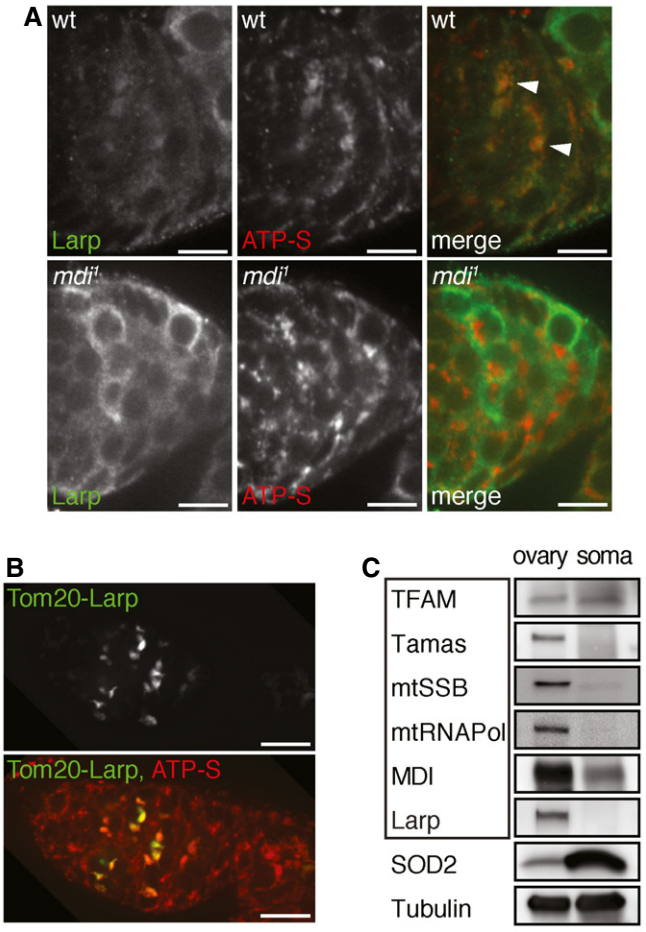

Figure EV3. Larp localization in ovaries and expression of mitochondrial proteins in ovary and somatic tissues.

A Wild-type and *mdi*^{1} germaria were stained for Larp (green) and ATP-S (red) to reveal mitochondria. Note that Larp closely associates with mitochondria in wt germarium (arrowheads). Mitochondria in *mdi*^{1} flies completely lack Larp staining. Scale bars, 10 μm.

B Tom20-LarpGFP (Tom20-Larp) fusion protein was expressed under the control of *nanos-gal4* in an *mdi*^{1} egg chamber that was stained with ATP-S (red) to mark mitochondria. Note that Tom20-Larp is concentrated around mitochondria (ATP-S) in *mdi*^{1} background. Scale bars, 10 μm.

C Western blots of several mitochondrial proteins in ovary and somatic tissues of wild-type flies. Boxed are mtDNA replication factors, including TFAM, mtDNA polymerase (Tamas), mitochondrial single-strand DNA binding protein (mtSSB), mitochondrial RNA polymerase (mtRNAPol), MDI, and Larp. Except for TFAM, most proteins required for mtDNA replication are upregulated in ovary mitochondria. Tubulin was used as a loading control.

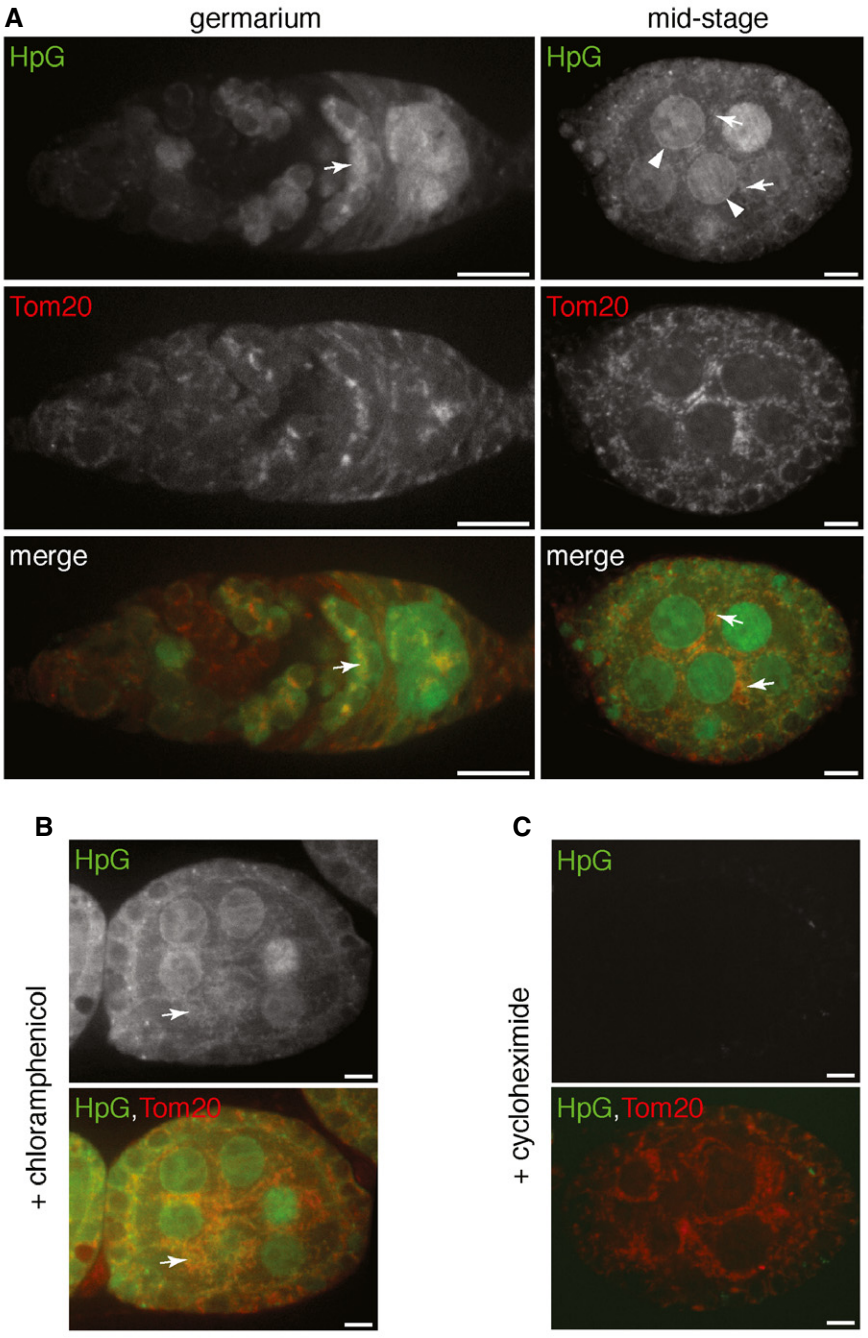

Figure EV4. Nascent protein synthesis in ovary visualized by HpG incorporation.

A Representative images of ovarioles expressing Tom20-mCherry that were incubated with a methionine analog, L-homopropargylglycine (HpG) for a pulse of 30 min to label the nascent protein synthesis. HpG was visualized with Alexa Fluor 488 through click-it chemistry. Alexa Fluor 488 signal of the nascent protein synthesis was sensitive to detergent wash used in immunostaining assay. Thus, Tom20-mCherry was used to mark mitochondria.

B Representative image of HpG incorporation in ovarioles in the presence of chloramphenicol that inhibits mitochondrial ribosomes specifically. Chloramphenicol has no impact on the HpG incorporation in mid-stage egg chamber.

C Representative image of HpG incorporation in ovarioles in the presence of cycloheximide that inhibits cytosolic ribosomes. Note that cycloheximide greatly reduces HpG signal.

Data information: Arrows point to the HpG signal associated with mitochondria. Arrowheads point to the HpG signal at perinuclear region. Scale bars in (A–C), 10 μm.

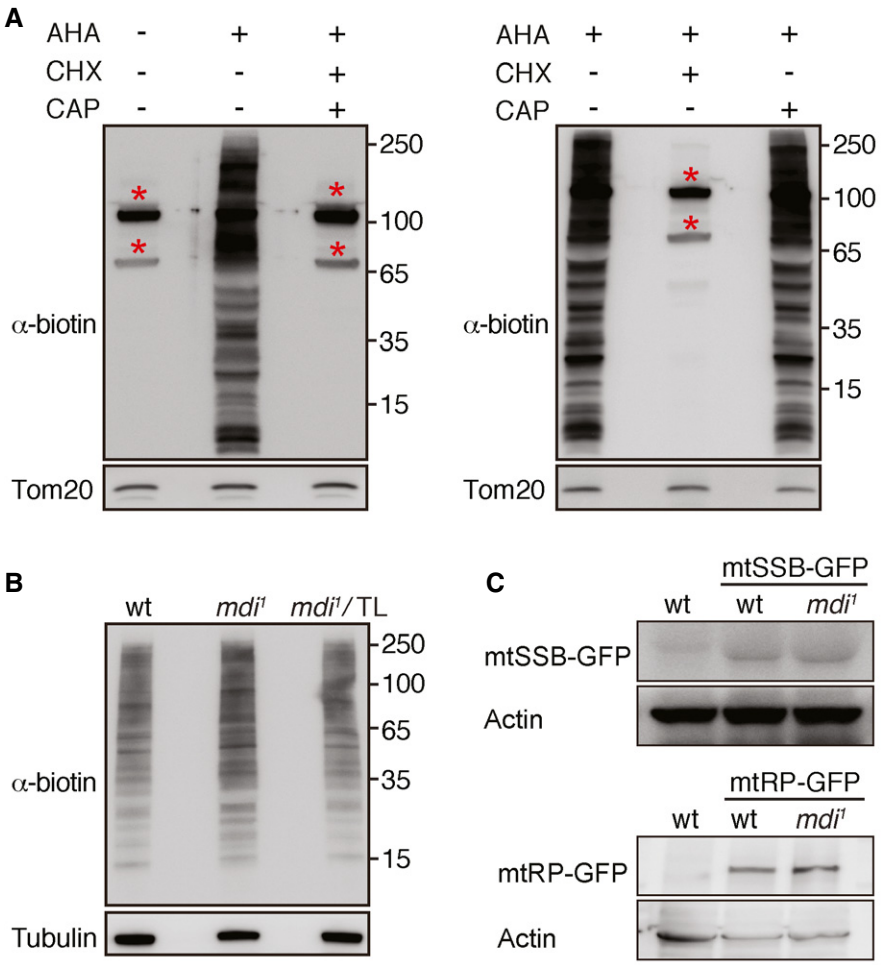

Figure EV5. Western blot analyses of nascent protein synthesis and steady-state protein levels in ovaries.

A Western blot analyses of nascent protein synthesis in the mitochondrial fraction of the ovary. The nascent protein synthesis was labeled by AHA incorporation and detected by anti-biotin antibody. Tom20 was used as a loading control. There were two strong bands (*) in the mitochondria fraction without the AHA incubation, indicating two endogenously biotinylated mitochondrial proteins. The AHA signal was mostly blocked in the presence of a cytosolic translation inhibitor, cycloheximide (CHX). Whereas chloramphenicol (CAP) has no impact on the HpG incorporation, indicating the nascent protein synthesis is mainly derived from cytosolic ribosomes associated with mitochondria.

B Western blot analyses of nascent protein synthesis in the cytosolic fraction of wt, *mdi*^{1}, and *mdi*^{1} expressing Tom20-Larp (*mdi*^{1}/TL) ovary. Tubulin was used as a loading control. The overall AHA signals indicating the nascent protein synthesis in the three genotypes are comparable.

C Western blot analyses of mtSSB-GFP and mtRNApol-GFP (mtRP-GFP) in wt and *mdi*^{1} ovary. Actin was used as a loading control.