Cardiolipin Affects the Supramolecular Organization of ATP Synthase in Mitochondria

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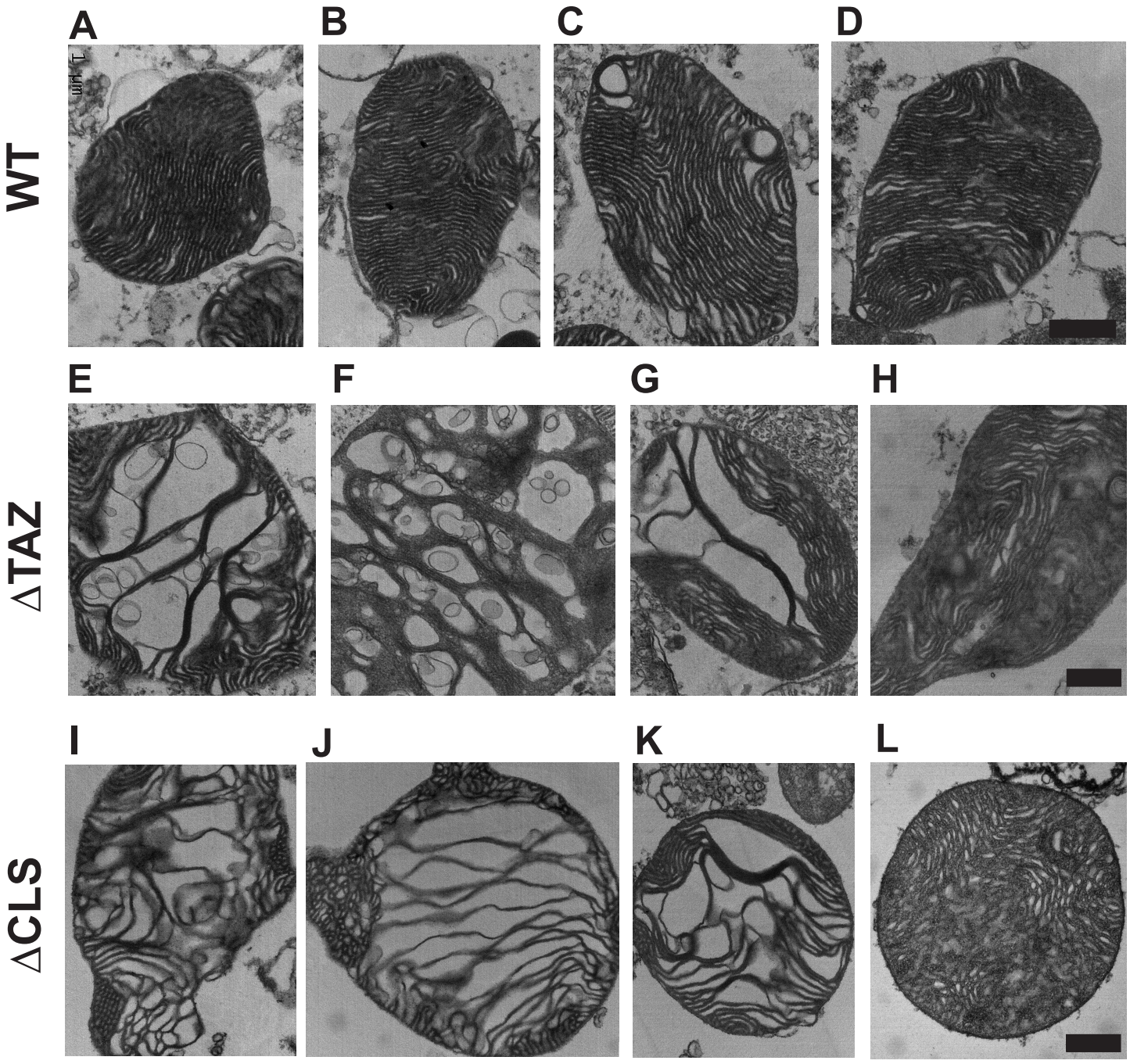
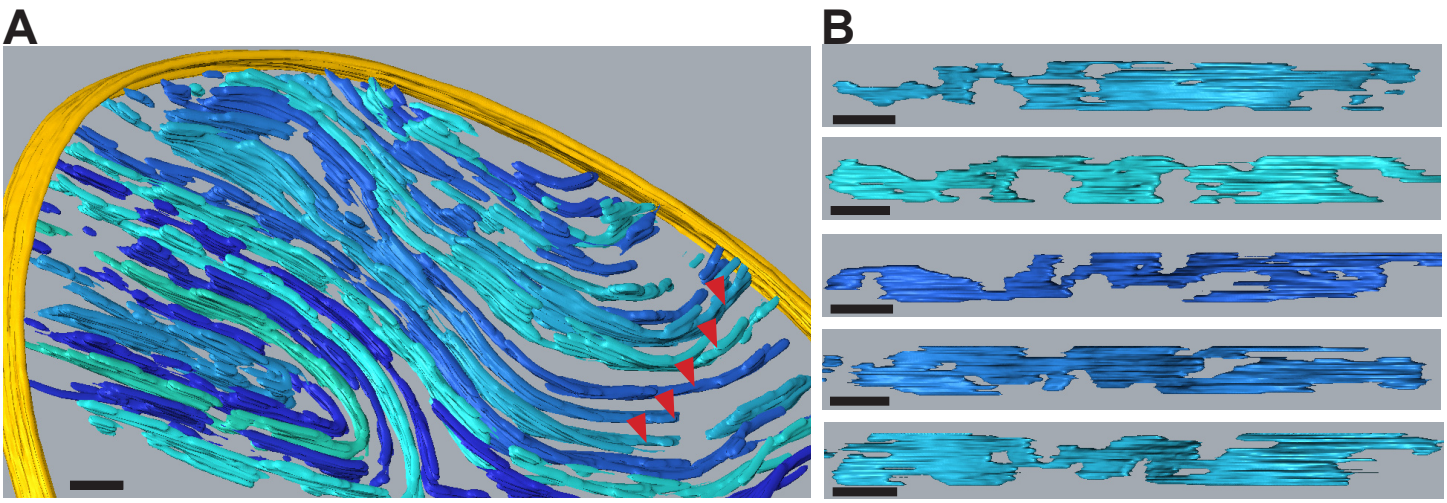
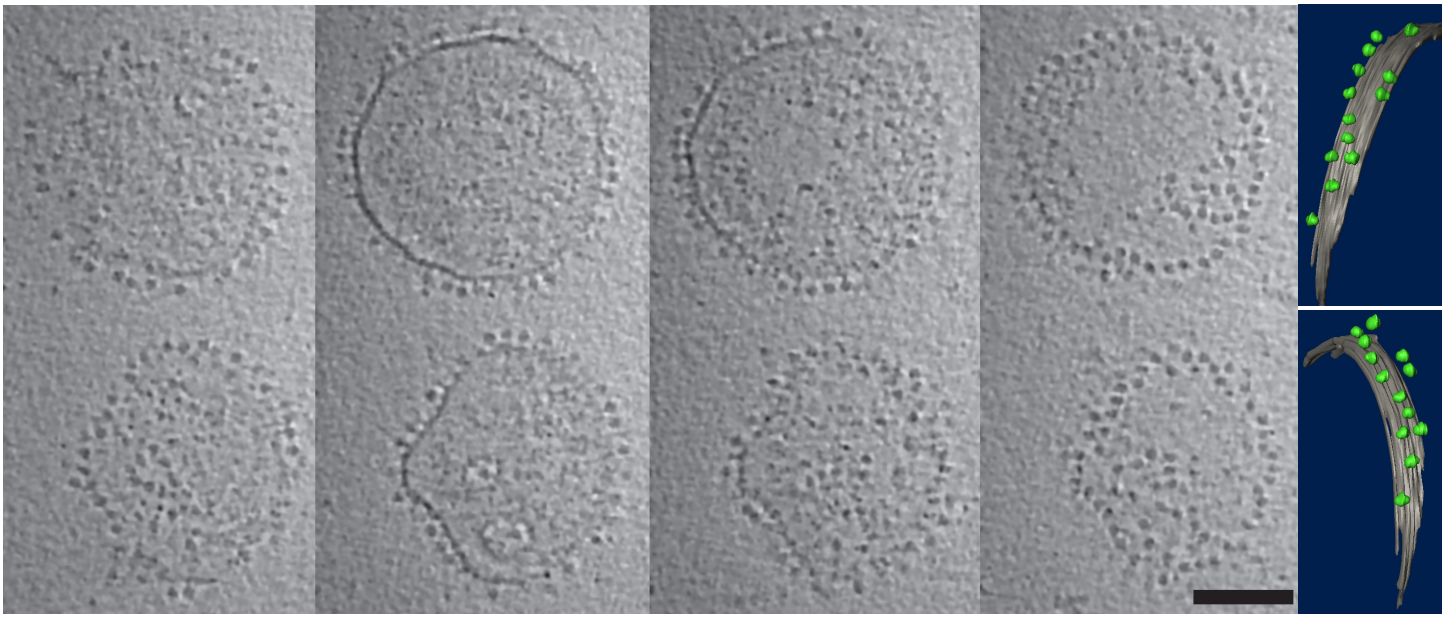
Supporting Material



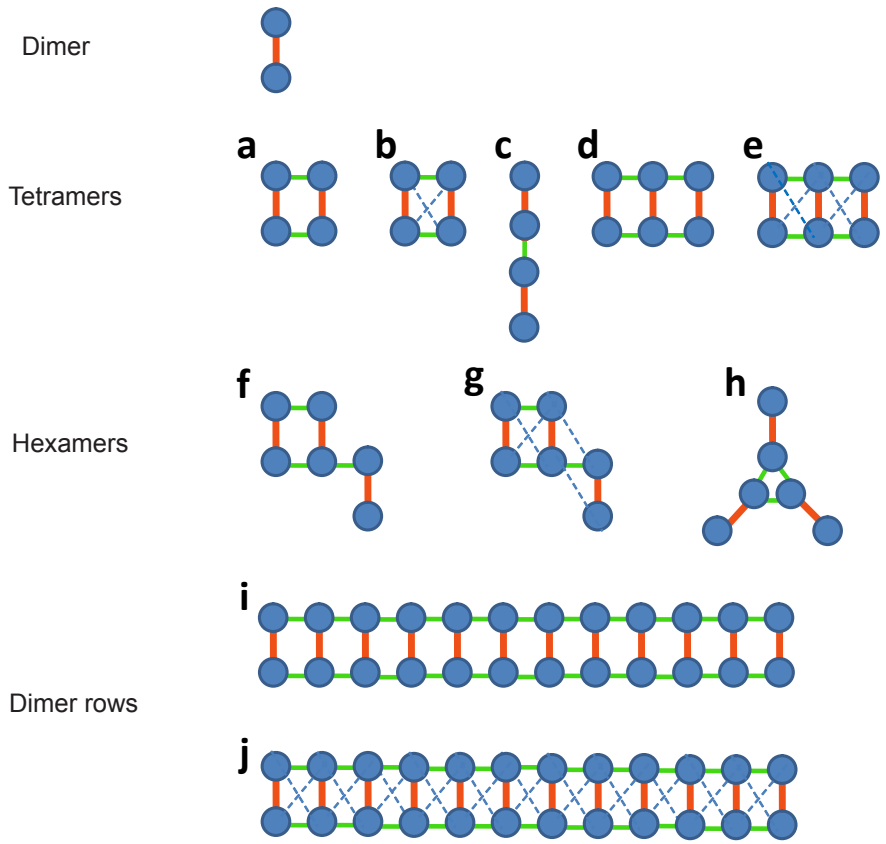
Supporting Figure S1. Electron micrographs of isolated mitochondria from Drosophila flight muscle. Mitochondria were isolated, chemically fixed, resin infiltrated, sectioned and stained with uranyl acetate and lead stain. Wild-type mitochondria (A-D) show the characteristic morphology of flight muscle mitochondria with high cristae density (19, 27). Preparations from the tafazzin mutant (ΔTAZ) contain both abnormal (E-G) and normal (H) mitochondria. Preparations from the cardiolipin synthase mutant (ΔCLS) also contain abnormal (I-K) and normal (L) mitochondria. Bars: 500 nm.



Supporting Figure S2. Ultrastructure of flight muscle mitochondria. Drosophila flight muscle was fixed and electron microscopic tomography was performed as described (20). (A) The 3-D model shows densely packed multi-lamellar cristae in shades of blue. The outer membrane is shown in yellow. (B) Single cristae, marked with red arrow heads in panel A, are shown in a different view. Cristae consist of flat sacs (50-250nm) interconnected by tubes. Bars: 100nm.

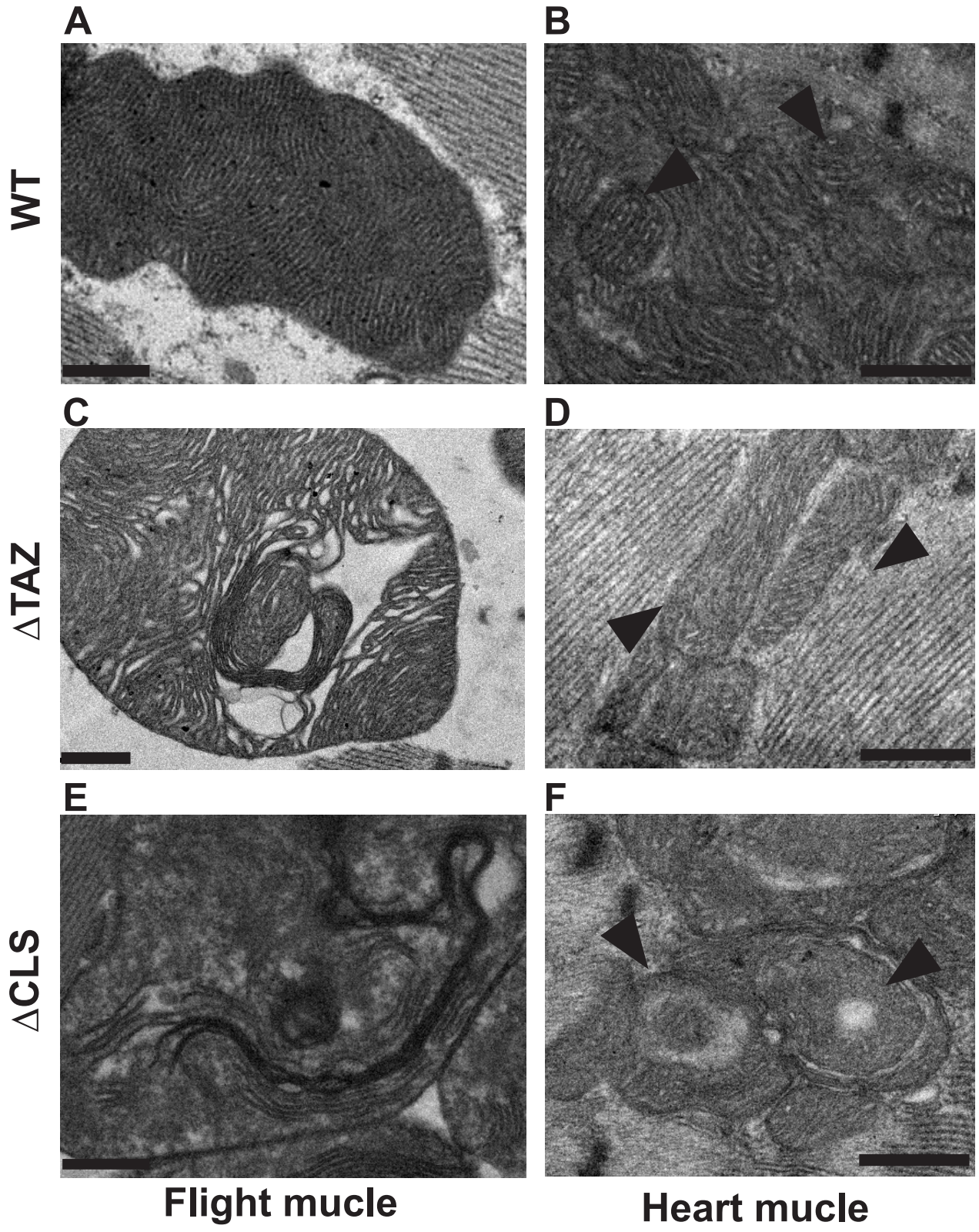


Supporting Figure S3. Slices of a tomogram of two membrane vesicles. Drosophila flight muscle mitochondria were isolated and processed for cryo-electron microscopy as described in Materials and Methods. Tomogram slices are arranged according to their position in the volume. Numerous F1 particles (diameter: 9 nm) are visible at the membrane surface. A 3-D model of a portion of the tomogram is shown on the right with the membrane in grey and selected F1 particles in green. Bar: 100 nm.

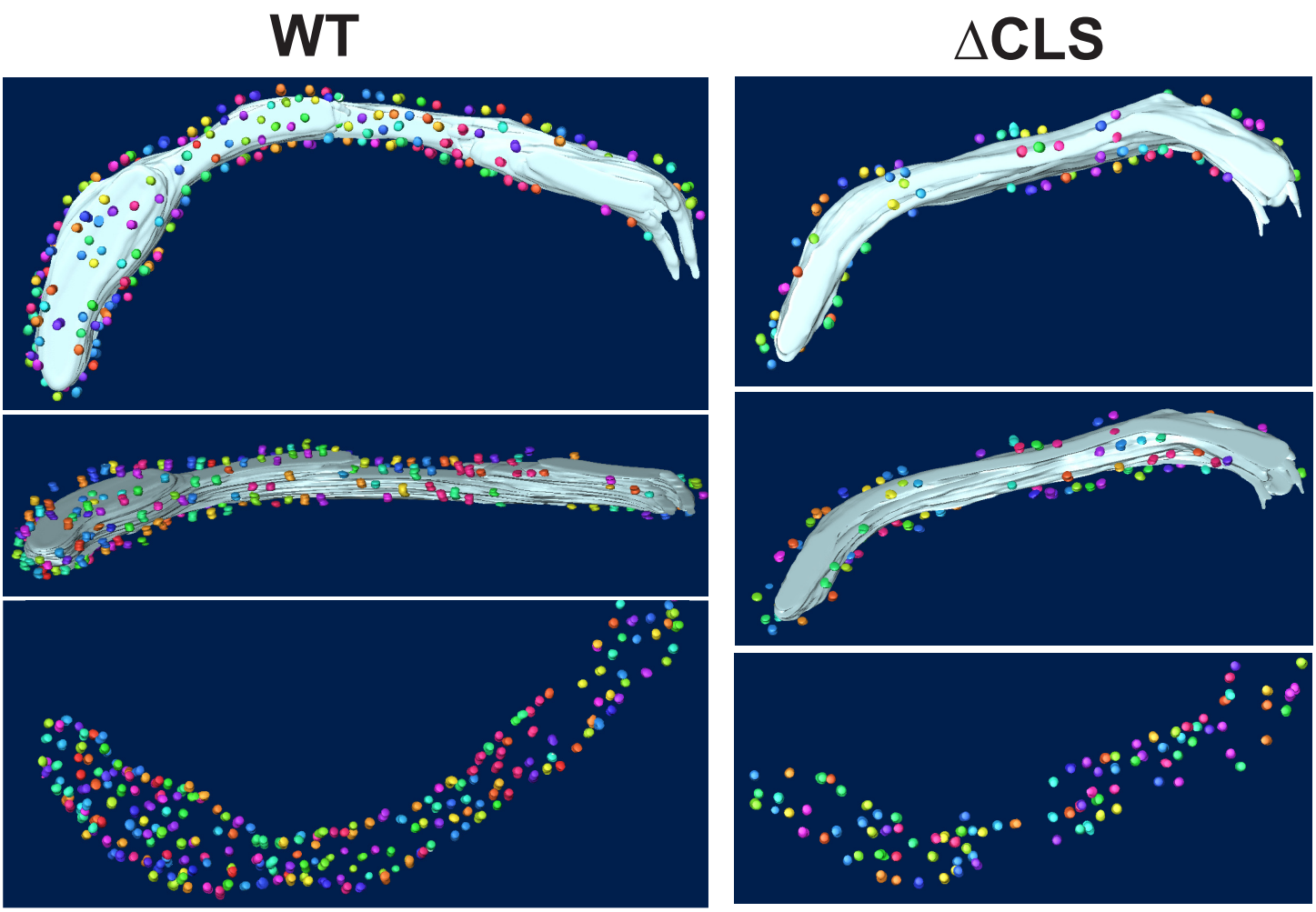


Type	Species	Average number of neighbors per F_1 particle		
		Total	Close neighbors (green lines)	Far neighbors (red lines and dashed lines)
Dimer		1	0	1
Tetramers	a b c	2 3 1½	1 1 ½	1 2 1
Hexamers	d e f g h	2⅓ 3⅔ 2 3⅓ 2	1⅓ 1⅓ 1 1 1	1 2⅓ 1 2⅓ 1
Dimer rows	i j	3 5	2 2	1 3

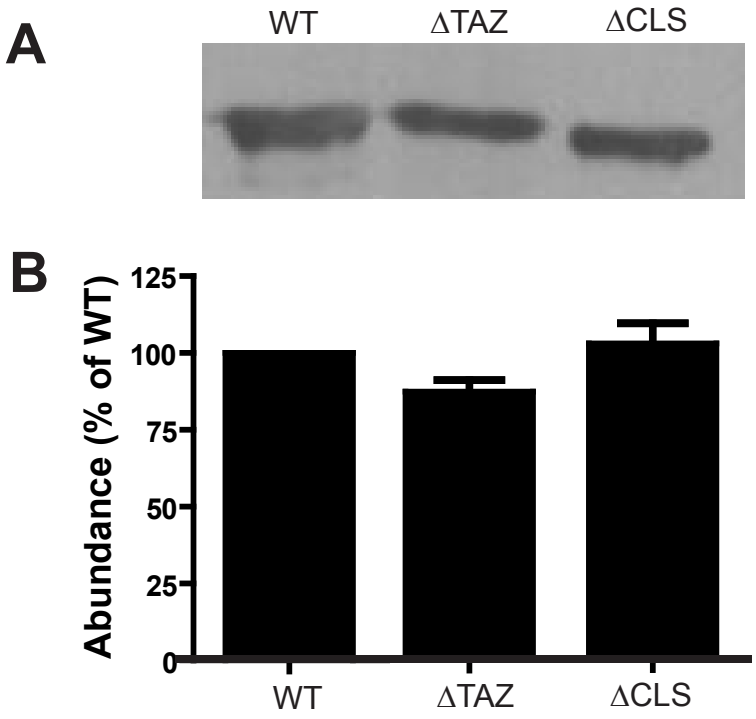
Supporting Figure S4. Idealized structural models of hypothetical assemblies of ATP synthase dimers. Dimers can associate to form various types of tetramers (a-c), hexamers (d-h), or multimers (i-j). In this figure, dimers are shown as two circles connected by a red line. Green lines indicate dimer-dimer interactions and blue dashed lines indicate that F1 particles in diagonal position are close enough to be considered neighbors. The experimental data suggest the presence of two close neighbors and two far neighbors per F1 particle, which is consistent with an intermediate structure between i and j.



Supporting Figure S5. Electron micrographs of mitochondria in situ. Drosophila thoraces were fixed and stained as described (20). The images show electron micrographs of flight muscle and heart muscle in wild-type flies (WT), tafazzin mutants (ΔTAZ), and cardiolipin synthase mutants (ΔCLS). Arrow heads point to mitochondria. In ΔTAZ, abnormal mitochondria were observed in flight muscle (C). In ΔCLS, abnormal mitochondria were observed in both flight muscle (E) and heart muscle (F). Bars: 500 nm.



Supporting Figure S6. Tubular fragments of Drosophila flight muscle mitochondria. The figure shows 3-D models of tomograms of tubular fragments from wild-type (WT) and cardiolipin synthase mutant (ΔCLS) mitochondria in different views. F1 particles are shown in random colors; the membrane surface is shown in grey. The surface concentration of ATP synthase is lower in ΔCLS compared to WT.



Supporting Figure S7. Abundance of ATP synthase in flight muscle mitochondria isolated from Drosophila strains, including wild-type (WT), cardiolipin synthase mutant (ΔCLS), and tafazzin mutant (ΔTAZ). (A) Western blot with monoclonal antibody to the α-subunit of ATP synthase (25 μg mitochondrial protein per lane). (B) Densitometry of four Western blot analyses. Results are expressed in percent of wild-type abundance.

Supporting Movie. 3-D model showing two parallel rows of ATP synthase dimers at the high-curvature edge of a membrane vesicle. Two parallel rows of dimers (one colored red, the other colored blue) are located on opposite sides of the edge of the disc-shaped vesicle. Dimers are colored in alternating shades of red or blue. F_1 particles on the flat surface of the vesicle are colored white and F_1 particles that could not be assigned to any dimer are colored yellow. The surface of the vesicle is colored grey.