1 Electron tomographic analysis reveals ultrastructural features of mitochondrial 2 cristae architecture which reflect energetic state and aging 3 Yi-fan Jiang^{1,2}, Shao-syuan Lin¹, Jing-min Chen¹, Han-zen Tsai¹, Tao-shih Hsieh¹, 4 and Chi-vu Fu¹* 5 ¹Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan 6 ²Graduate Institute of Molecular and Comparative Pathobiology, School of 7 8 Veterinary Medicine, National Taiwan University, Taipei, Taiwan 9 * Corresponding author 10 11 **Supplementary information** 12 Figure S1. Serial-section electron tomography of a single mitochondrion 13 (A) 2D micrographs and (B) 3D tomographic slices of serial sections covering an 14 entire volume of a mitochondrion. (C) The joint serial-section tomograms as viewed 15 from the z-axis. Of note, material losses during tissue sectioning left gaps between jointed tomograms. 16 17 Figure S2. Western-blot analysis of R_{WT} and R_{M1L} 18

Western-blot analysis of total protein expression of R_{WT} and R_{MIL} at week 1, 4, 7.

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21 Figure S3. Global disintegration of mitochondrial cristae in aged WT Drosophila 22 (A) Immunofluorescent staining of mitochondria at week 4, 7, 10 and 12 showing larger mitochondria as flies aged. (B) Thin-section EM micrographs of WT IFM at 23 week 10 and 12 showing mitochondrial cristae swelling, vacuolation and 24 25 disintegration. Scale bar 20 µm (A), 0.5 µm (B). 26 27 Figure S4. Mitochondrial protein content in aged WT Drosophila 28 (A) Immuno-EM analysis of ATP synthase, (B) PDHA1 and (C) SOD2 at week 4 and 29 12. (D) Western-blot analysis of total protein expression at week 4, 7, 10. Scale bar 30 $0.5 \mu m$. 31 32 Movie S1. Serial-section electron tomography and segmentation of IFM 33 mitochondrion showing integrated cristae network in 3D 34 35 Movie S2. Serial-section electron tomography and segmentation of IFM 36 mitochondrion showing bidirectional matrix confluency in 3D 37 38 Movie S3. Serial-section electron tomography and segmentation of a mitochondrion 39 with swirling membranes showing an onion-like core in 3D 40

Fig S1

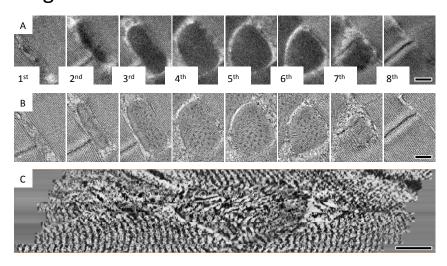


Fig S2

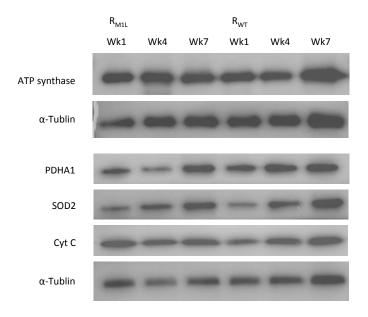


Fig S3

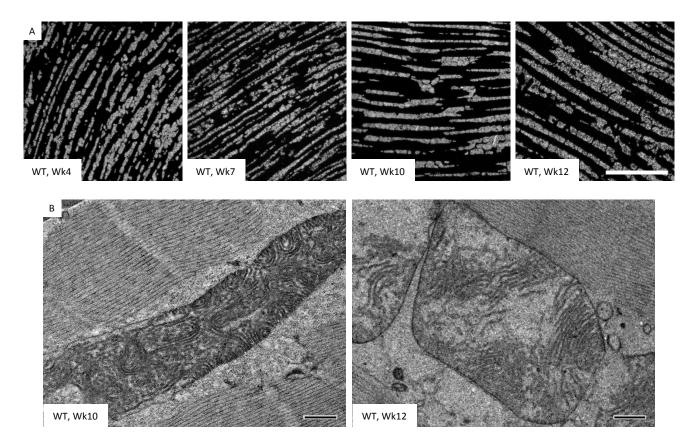


Fig S4

