Expanded View Figures

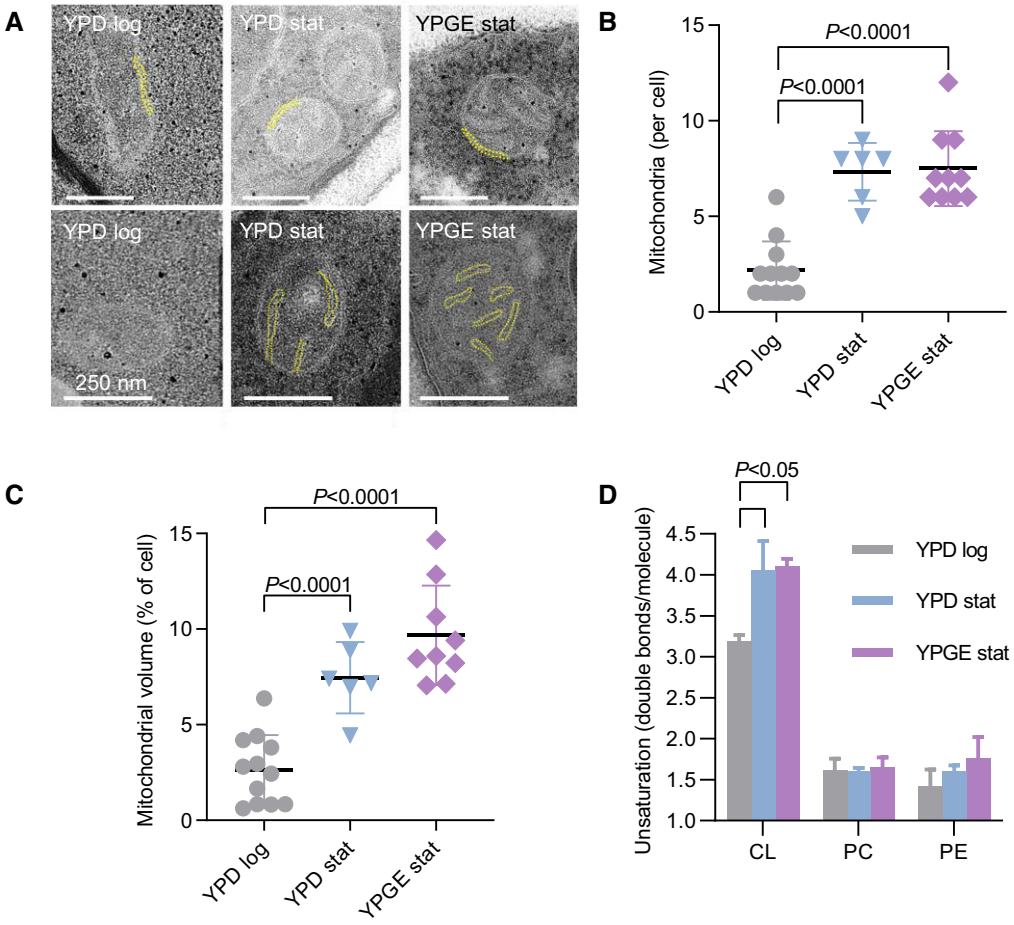

Figure EV1. Stationary growth increases the density of cristae, the abundance of mitochondria, and the unsaturation of cardiolipin (CL), but not the unsaturation of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in *S. cerevisiae*.

Cultures of wild-type *S. cerevisiae* were harvested during logarithmic growth in glucose (YPD log), stationary growth in glucose (YPD stat), and stationary growth in glycerol/ethanol (YPGE stat).

A The mitochondrial ultrastructure was analyzed by transmission electron microscopy. The yellow labels show the double membrane of mitochondria (OM and boundary IM) in the upper panels and mitochondrial cristae (IM) in the lower panels. Scale bars have a length of 250 nm.

B, C The number of mitochondrial cross-sections and the mitochondrial volume were quantified in randomly collected electron micrographs. Multiple micrographs were analyzed per biological replica.

D Lipids were analyzed by mass spectrometry and the number of double bonds per molecule was calculated from the compositions of CL, PC, and PE.

Data information: The graphs show biological replicas ($N = 3$), mean values, and standard deviations. Means were compared by Student's *t*-test.

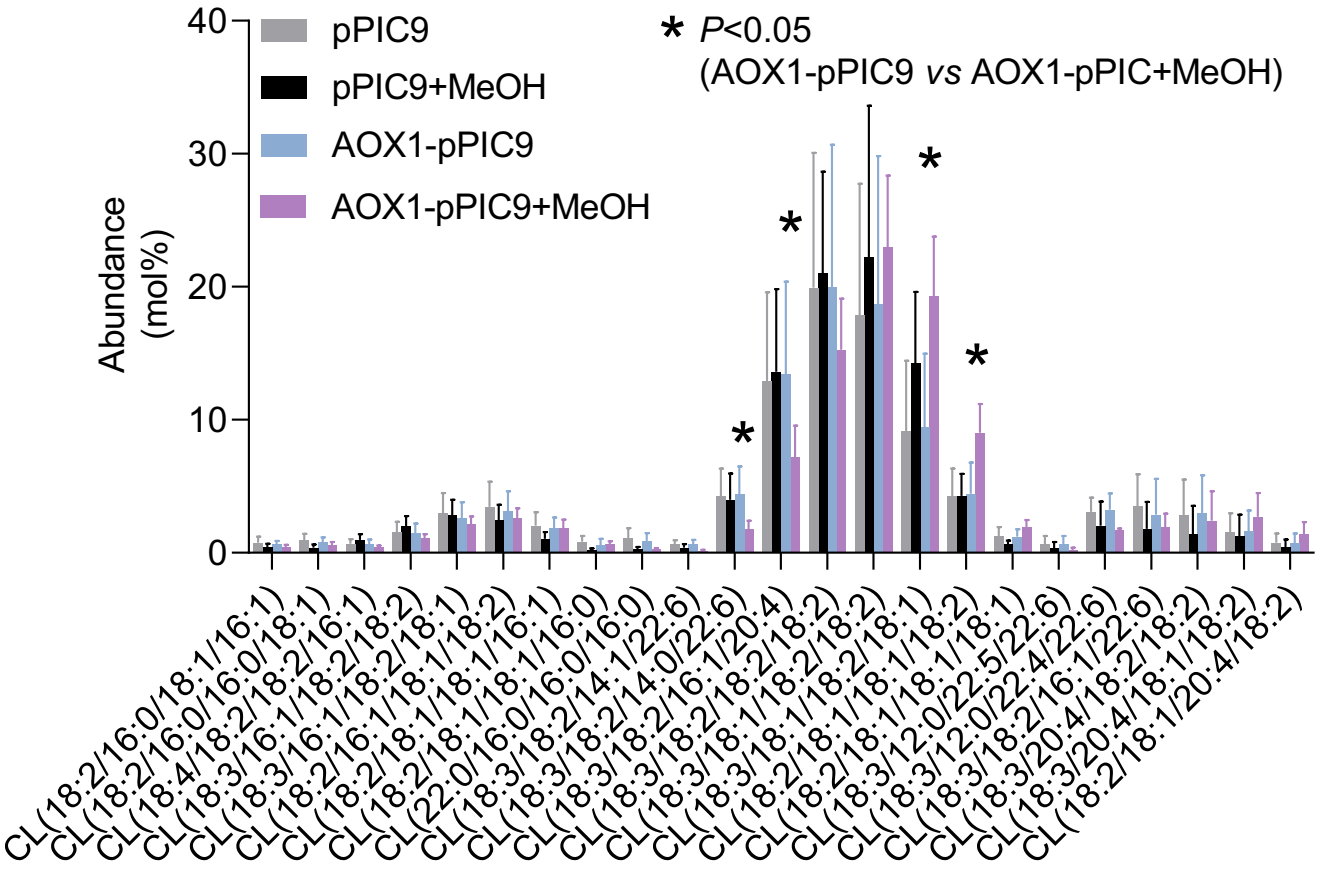

Figure EV2. AOX expression alters the molecular species composition of CL in *P. pastoris*.

Cells were transformed with alternative oxidase (AOX1) inserted into pPIC9 vector DNA, which confers methanol-inducible overexpression of AOX1. Cells were grown in MGY medium either in the presence or absence of 1% methanol (MeOH). The molecular species composition of CL was analyzed. Only species > 0.5 mol% are shown. The graph shows biological replicas ($N = 4$), mean values, and standard deviations. Means were compared by Student's *t*-test.

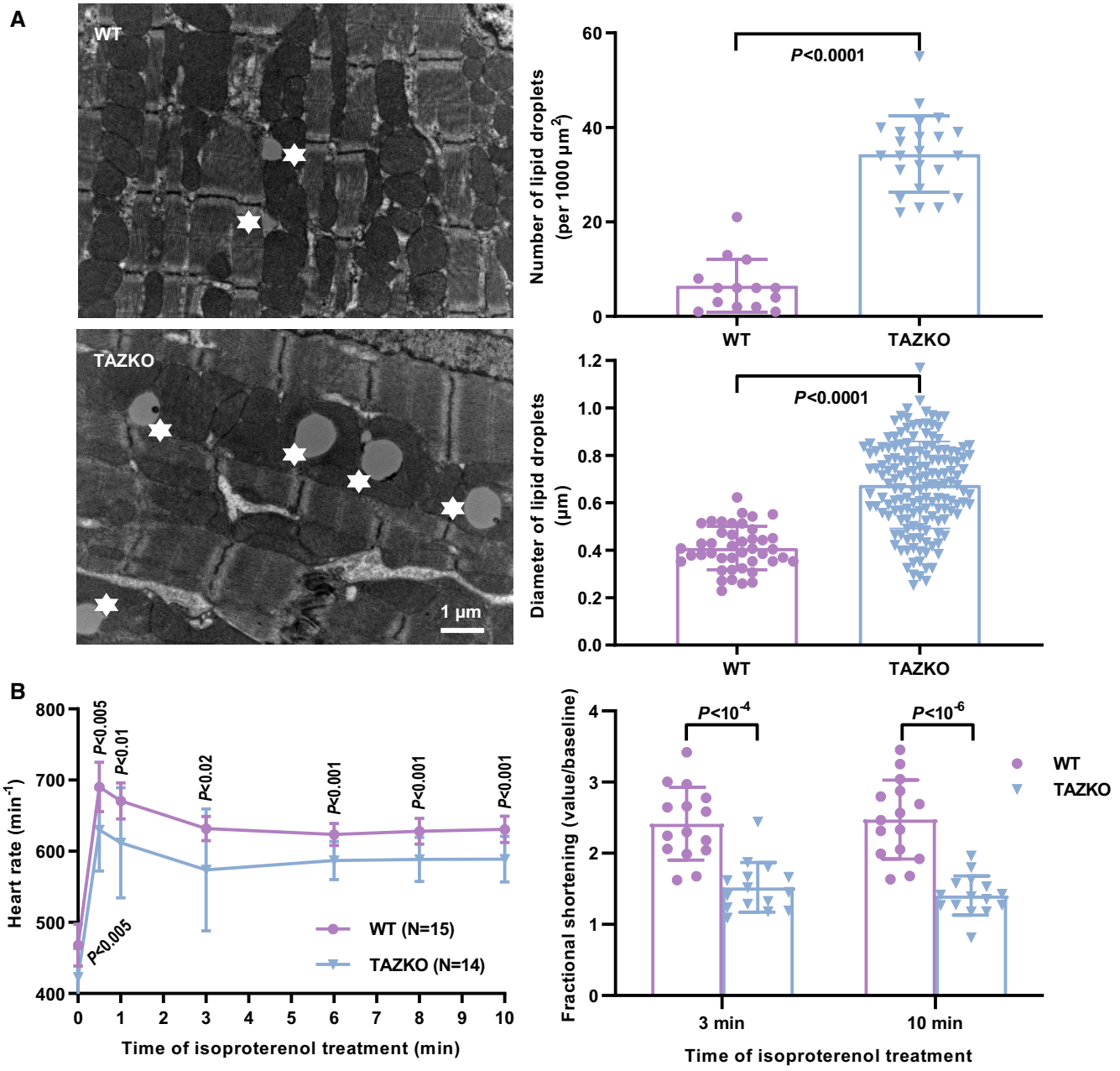

Figure EV3. TAZKO causes cardiomyopathy in mice.

TAZKO mice (5 months old) were compared to littermate controls (WT).

A Heart tissue was analyzed by quantitative electron microscopy. Lipid droplets are marked by asterisks. Graphs show mean values and standard deviations. Multiple micrographs were analyzed in three biological replicas.

B Anesthetized mice were subjected to a pharmacologic stress test by isoproterenol infusion. Cardiac performance was analyzed by echocardiography.

Data information: The graphs show mean values and standard deviations of the indicated number of biological replicas. Means were compared by Student's *t*-test.

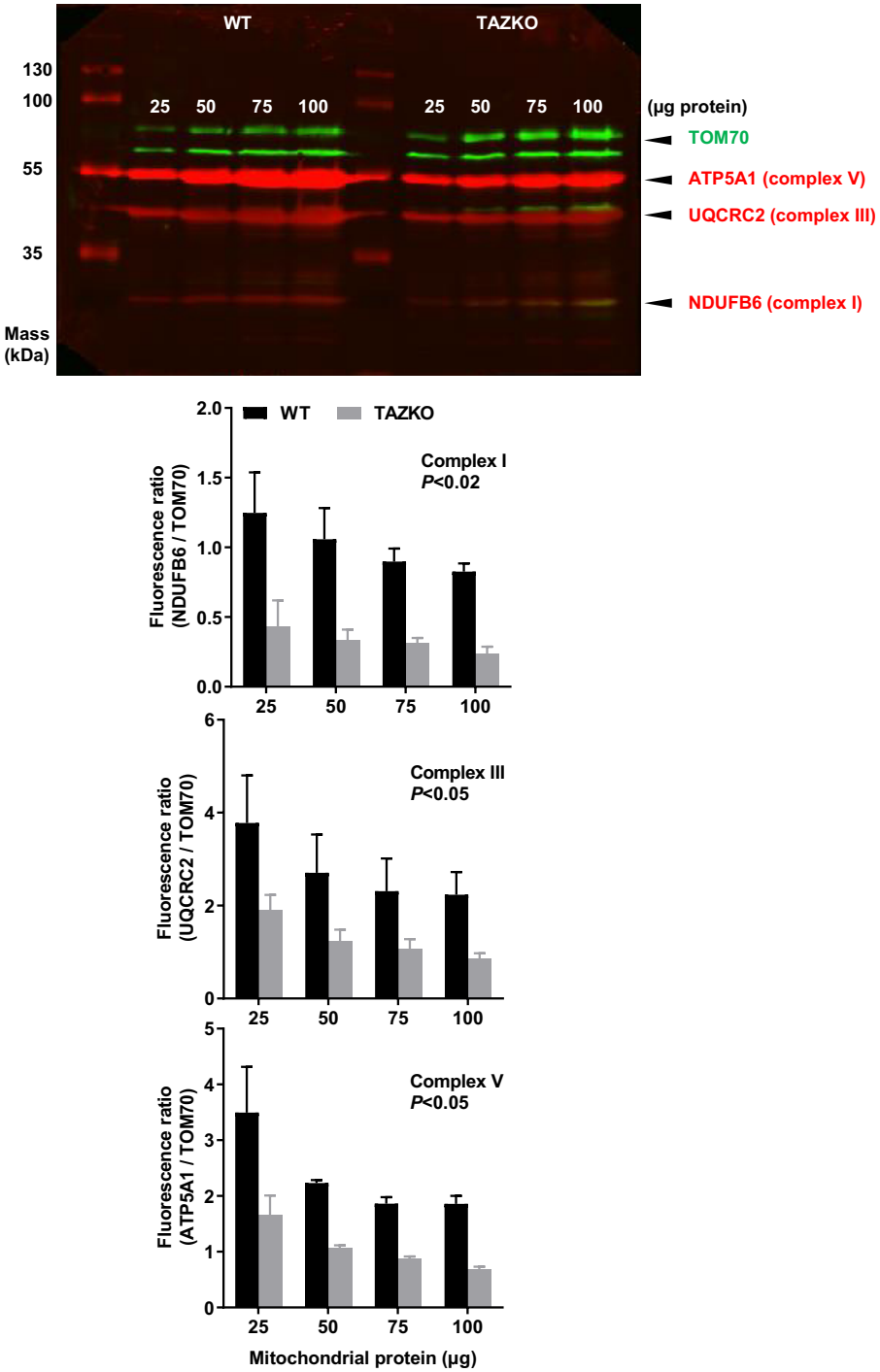

Figure EV4. TAZKO reduces the abundance of OXPHOS complexes I, III, and V in heart mitochondria.

Heart mitochondria were isolated from 5 months old mice (WT and TAZKO). Different amounts of mitochondria (25–100 µg protein) were analyzed by quantitative Western blotting with fluorescence-labeled secondary antibodies. The graphs show mean values and standard deviations of three biological replicas. Means were compared by Student's *t*-test.

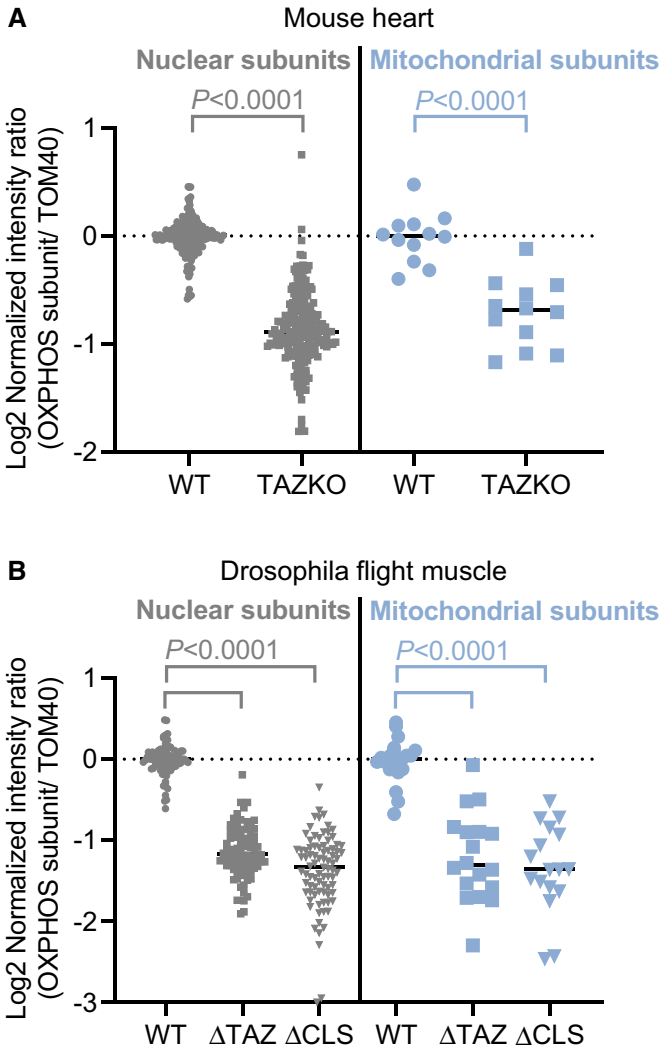

Figure EV5. CL deficiency reduces the abundance of OXPHOS subunits encoded by nuclear and mitochondrial DNA.

A Hearts were harvested from WT or TAZKO mice at the age of 5 months and analyzed by label-free quantitative proteomics.

B Indirect flight muscles were harvested from adult flies with mutations in the tafazzin gene (ΔTAZ) or the CL synthase gene (ΔCLS) or from wild type (WT) and analyzed by label-free quantitative proteomics.

Data information: The graphs show individual and mean values. Data represent 73 nuclear and four mitochondrial OXPHOS subunits (A) or 28 nuclear and seven mitochondrial OXPHOS subunits (B) of three biological replicas each. Means were compared by Student's *t*-test.