

Supplementary Materials for

Cardiolipin, conformation, and respiratory complex-dependent oligomerization of the major mitochondrial ADP/ATP carrier in yeast

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This PDF file includes:

Figs. S1 to S4

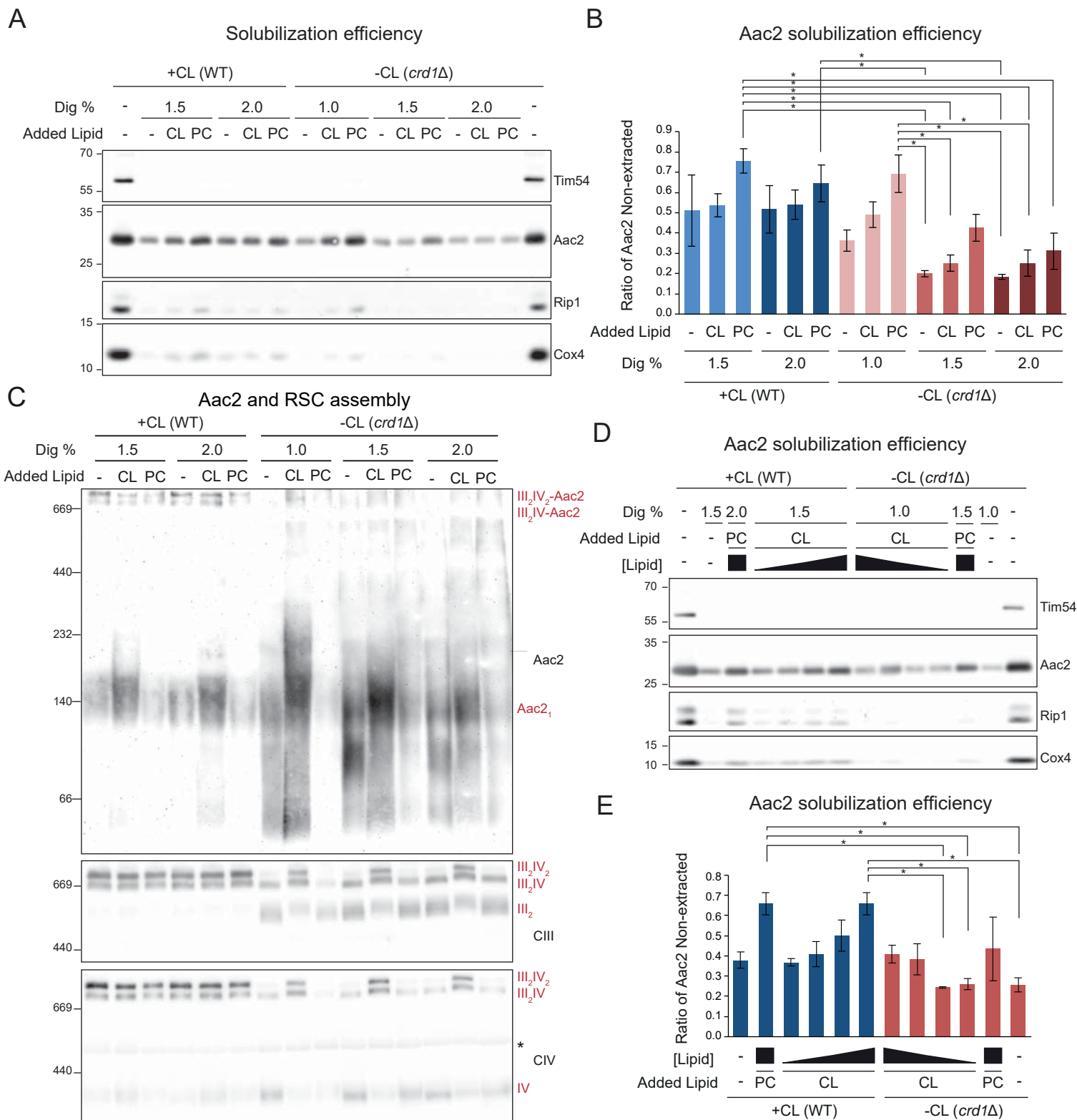


Figure S1. Aac2 solubilization efficiency is impacted by the presence of endogenous CL and the addition of exogenous PC. (A) Mitochondria from WT or *crd1Δ* yeast were mock-treated (-) or supplemented with CL or PC at 1.5:10 lipid/protein (g/g) ratio prior to solubilization at the indicated final concentration (% w/v) of digitonin. Extracted and non-extracted proteins were separated by centrifugation. Non-extracted proteins in the pellet were resolved by SDS-PAGE and immunoblotted as indicated. Non-extracted Mitochondria (20 μ g) were loaded on either side of the gel. (B) The ratio of Aac2 not extracted by digitonin in each sample was determined relative to non-extracted mitochondria. (mean \pm S.E.M. for n=3 independent experiments). Statistical comparisons (* $P \leq 0.05$) were performed by one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. (C) In parallel, the mitochondrial extracts from (A) were resolved by 6-16% BN-PAGE and immunoblotted for Aac2 (top panel), complex III (CIII, Rip1; middle panel), or complex IV (CIV, Cox4; bottom panel). (n=3) Aac2₁, Aac2 monomer; III₂IV₂-Aac2 and III₂V-Aac2, Aac2 associated with large and small RSCs, respectively. (D) Non-extracted proteins following solubilization of mitochondria provided exogenous CL or PC, as listed, were resolved by SDS-PAGE and immunoblotted as indicated. Non-extracted Mitochondria (20 μ g) were loaded on either side of the gel. The corresponding BN-PAGE of solubilized extracts are shown in Fig. 1A and fig. S2A. (E) The ratio of Aac2 not extracted by digitonin in each sample was determined relative to non-extracted mitochondria. (mean \pm S.E.M. for n=3 independent experiments). Statistical comparisons (* $P \leq 0.05$) were performed by one-way analysis of variance (ANOVA) with Tukey's multiple comparison test.

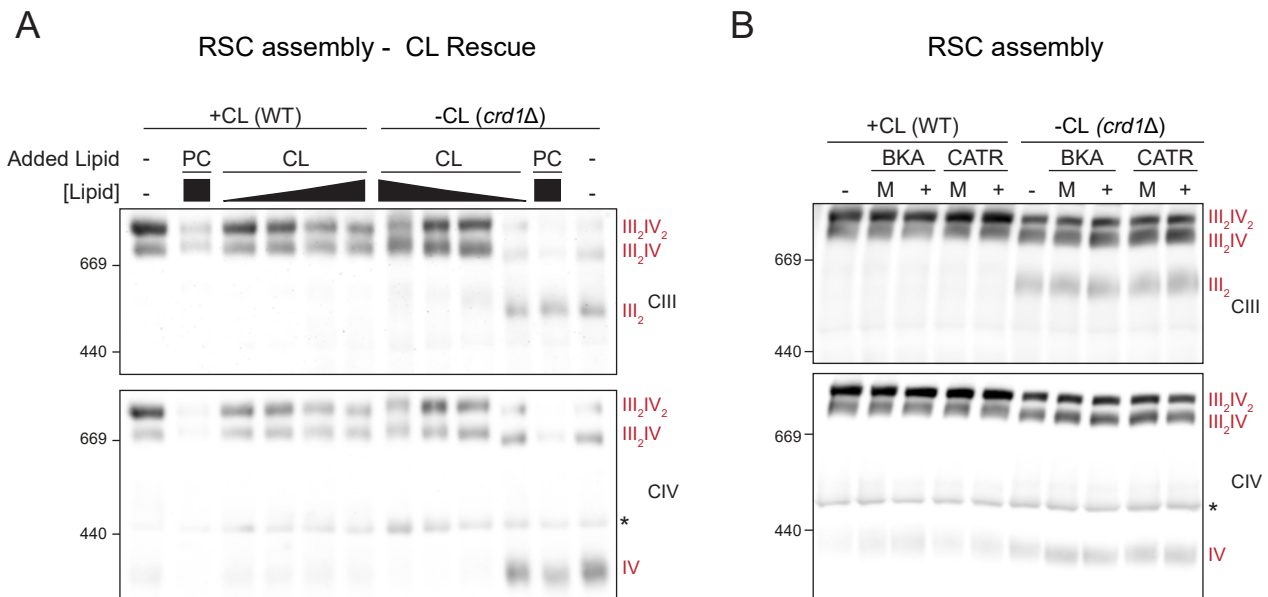


Figure S2. RSC assembly is restored by CL but not Aac2 stabilizing inhibitors. (A) Mitochondria from WT or *crd1Δ* yeast were supplemented with PC or increasing amounts of CL (relative amount added indicated) prior to solubilizing with an optimized concentration of digitonin to decrease differences in IM protein solubilization efficiency: 1.0% (w/v) for mock (-) or CL-treated *crd1Δ* mitochondria, 1.5% (w/v) for mock (-) or CL-treated WT mitochondria and PC-treated *crd1Δ* mitochondria, and 2.0% (w/v) for PC-treated WT mitochondria. Mitochondrial extracts were resolved by 6-16% BN-PAGE and immunoblotted for complex III (CIII, Rip1; *top panel*) or complex IV (CIV, Cox4; *bottom panel*). (n=3) **(B)** WT and *crd1Δ* mitochondria (100 μg), mock-treated (M) or instead incubated with BKA (10 μM) or CATR (40 μM), were solubilized with 1.5% (w/v) digitonin, resolved by 6-16% BN-PAGE, and immunoblotted for complex III (CIII, Rip1; *top panel*) or complex IV (CIV, Cox4; *bottom panel*). (n=3)

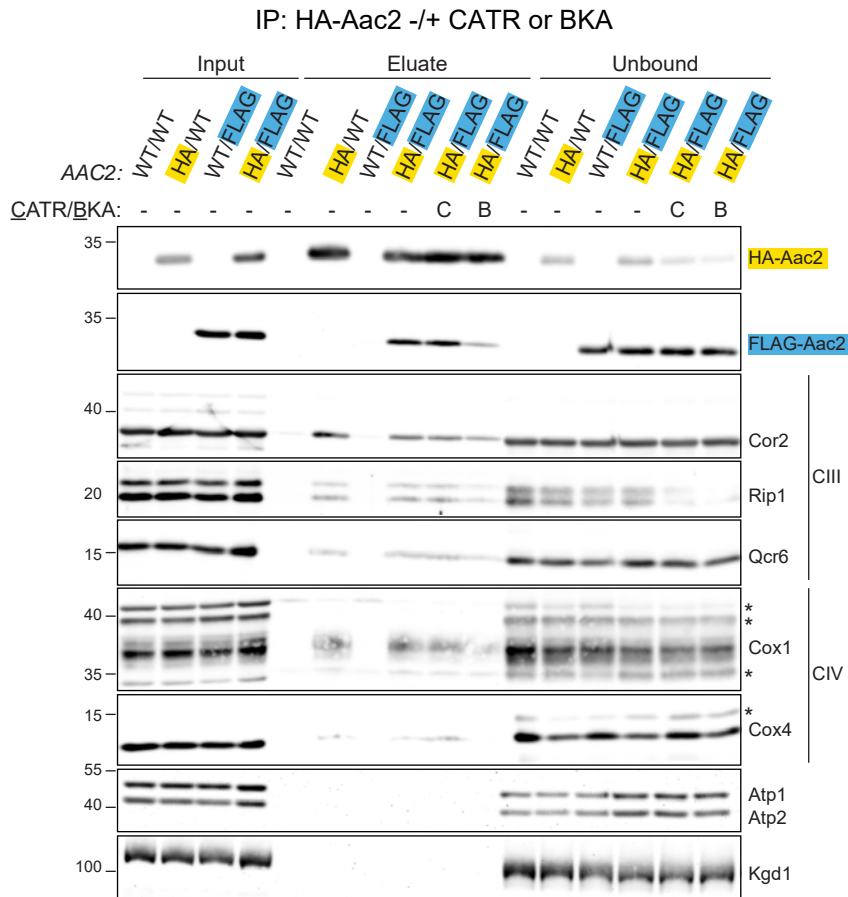


Figure S3. Aac2 oligomerization is conformation-sensitive. Mitochondria (250 μ g) from the indicated CL-producing strains pre-incubated with CATR (40 μ M) or BKA (10 μ M) as listed, were solubilized with 1.5% (w/v) digitonin and HA-Aac2 immunoprecipitated using anti-HA resin. The presence of co-purified FLAG-Aac2 and subunits of complexes III (Cor1, Cor2, Rip1, and Qcr6) and IV (Cox1, Cox4) was determined by immunoblotting; Atp1, Atp2 and Kgd1 served as controls. *, nonspecific bands. 4% of input (mitochondria) and unbound (flow through following HA

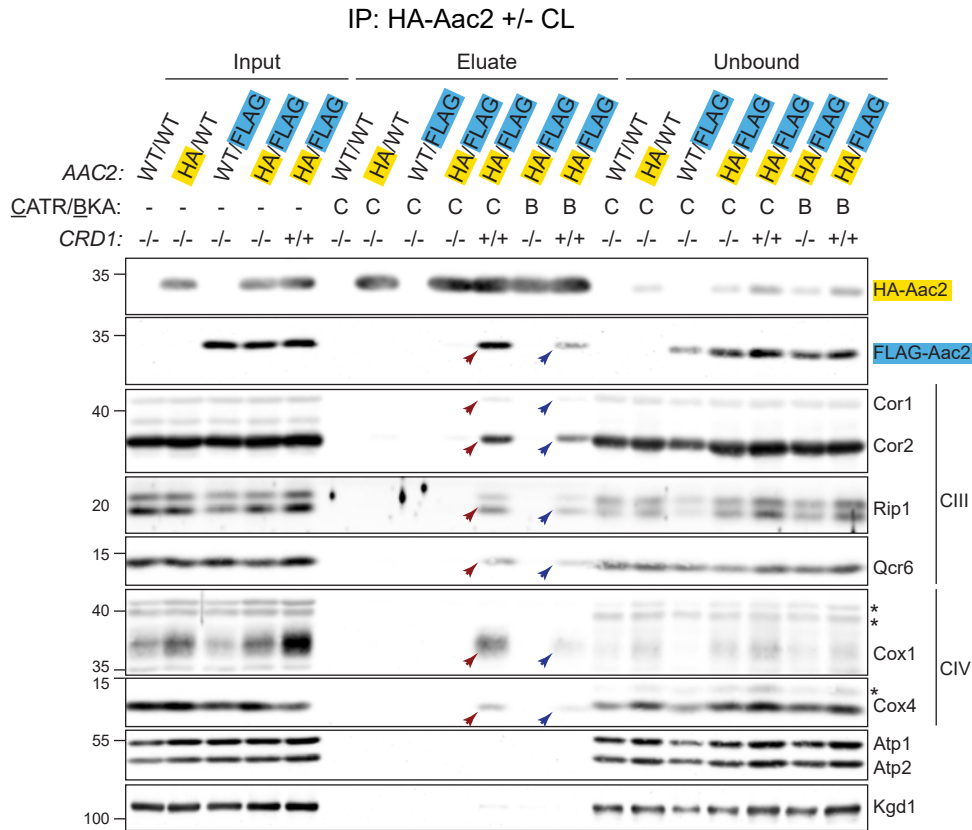


Figure S4. CL-dependent Aac2 oligomerization cannot be rescued with protein-stabilizing Aac2 inhibitors. WT or *crd1* Δ mitochondria (250 μ g), pre-incubated with CATR (40 μ M) or BKA (10 μ M) as listed, were solubilized with 1.5% (w/v) digitonin and HA-Aac2 immunoprecipitated using anti-HA resin. The presence of co-purified FLAG-Aac2 and subunits of complexes III (Cor1, Cor2, Rip1, and Qcr6) and IV (Cox1, Cox4) was determined by immunoblotting; Atp1, Atp2 and Kgd1 served as controls. *, nonspecific bands. Proteins that co-purified with HA-Aac2 when CL-containing mitochondria were pre-incubated with CATR (*red arrowheads*) or BKA (*blue arrowheads*) are marked. 4% of input (mitochondria) and unbound (flow through following HA immunoprecipitation) was analyzed. (n=4)