# Supplemental Materials

***Molecular Biology of the Cell***

Singhal et al.

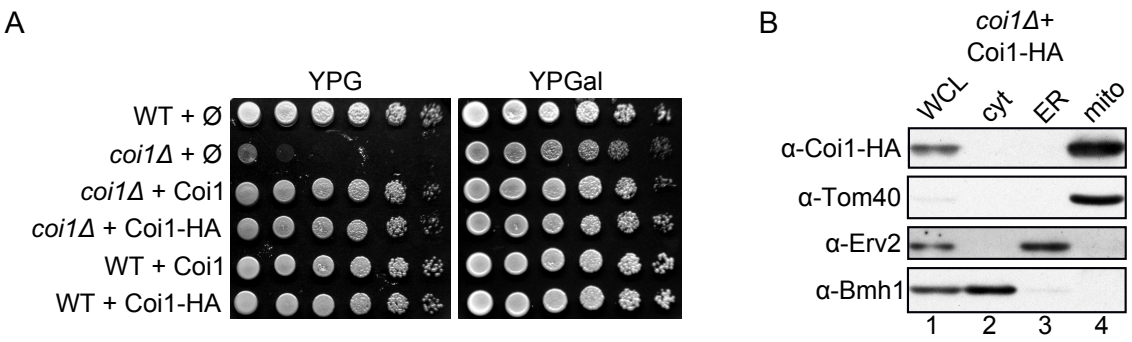


**Figure S1. Plasmids encoding Coi1 and Coi1-HA can complement the growth phenotype of *coi1Δ* cells. (A)** Wild type and *coi1Δ* cells were transformed with an empty plasmid or with a plasmid encoding either Coi1 or Coi1-HA. Cell were tested at 30°C by drop dilution assay on rich medium containing either fermentable carbon source (YPGal) or the non-fermentable carbon source glycerol (YPG).**(B)** Coi1-HA is localized to mitochondria. Whole-cell lysate (WCL) and fractions corresponding to cytosol (cyt), light microsomal fractions (ER) and mitochondria (mito) from *coi1Δ* cells expressing Coi1-HA were analyzed by SDS-PAGE and immunodecoration with antibodies against Coi1, Tom40 (mitochondrial protein), Erv2 (ER protein), and Bmh1 (cytosolic protein).

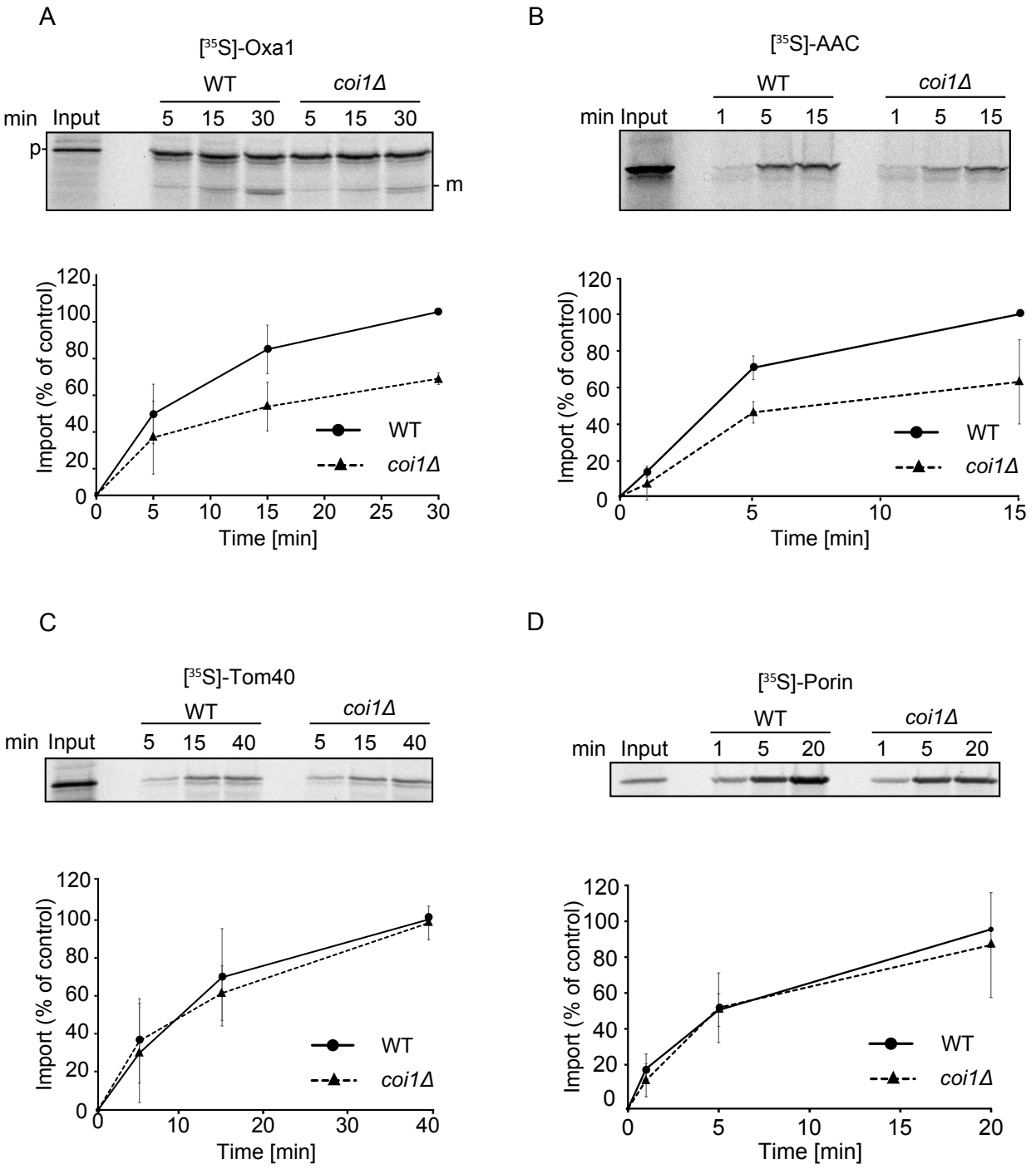


**Figure S2. The absence of Coi1 affects the import of several mitochondrial inner membrane proteins.** Radiolabeled precursors of Oxa1 **(A)**, AAC **(B)**, Tom40 **(C)** and Porin **(D)** were imported for the indicated time periods into mitochondria isolated from either wild type or *coi1Δ* cells. At the end of the import reactions, mitochondria were re-isolated and treated with proteinase K to remove non-imported material. Imported proteins were analyzed by SDS-PAGE and autoradiography. The input lane represents 20% of the radiolabelled protein used in each import reaction. All the experiments were prefomed in three independent repeats. Bands from three independent experiments were quantified and the amount of protein imported for the longest time period into control organelles was set to 100%.

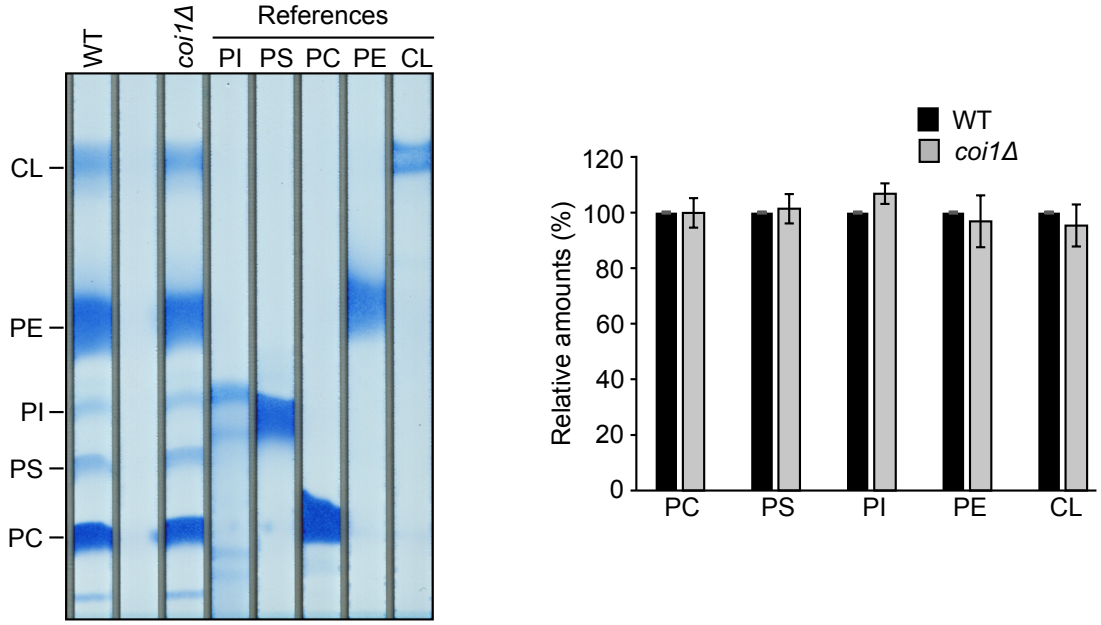


**Figure S3. Loss of Coi1 has no effect on the phospholipid composition of mitochondria.**
**(A)** Lipid extracted from highly pure mitochondria from either wild type or *coi1Δ* cells were analysed by thin layer chromatography. In parallel standards of the various phospholipids were analysed for comparison. The phospholipids were stained by molybdenum blue. **(B)** Bands corresponding to the various phospholipids groups were quantified and the amount of each phospholipid species in control mitochondria was set to 100% (n=3). Cadiolipin (CL); Phosphatidylethanolamine (PE); Phosphatidylinositol (PI); Phosphatidylserine (PS); Phosphatidylcholine (PC).

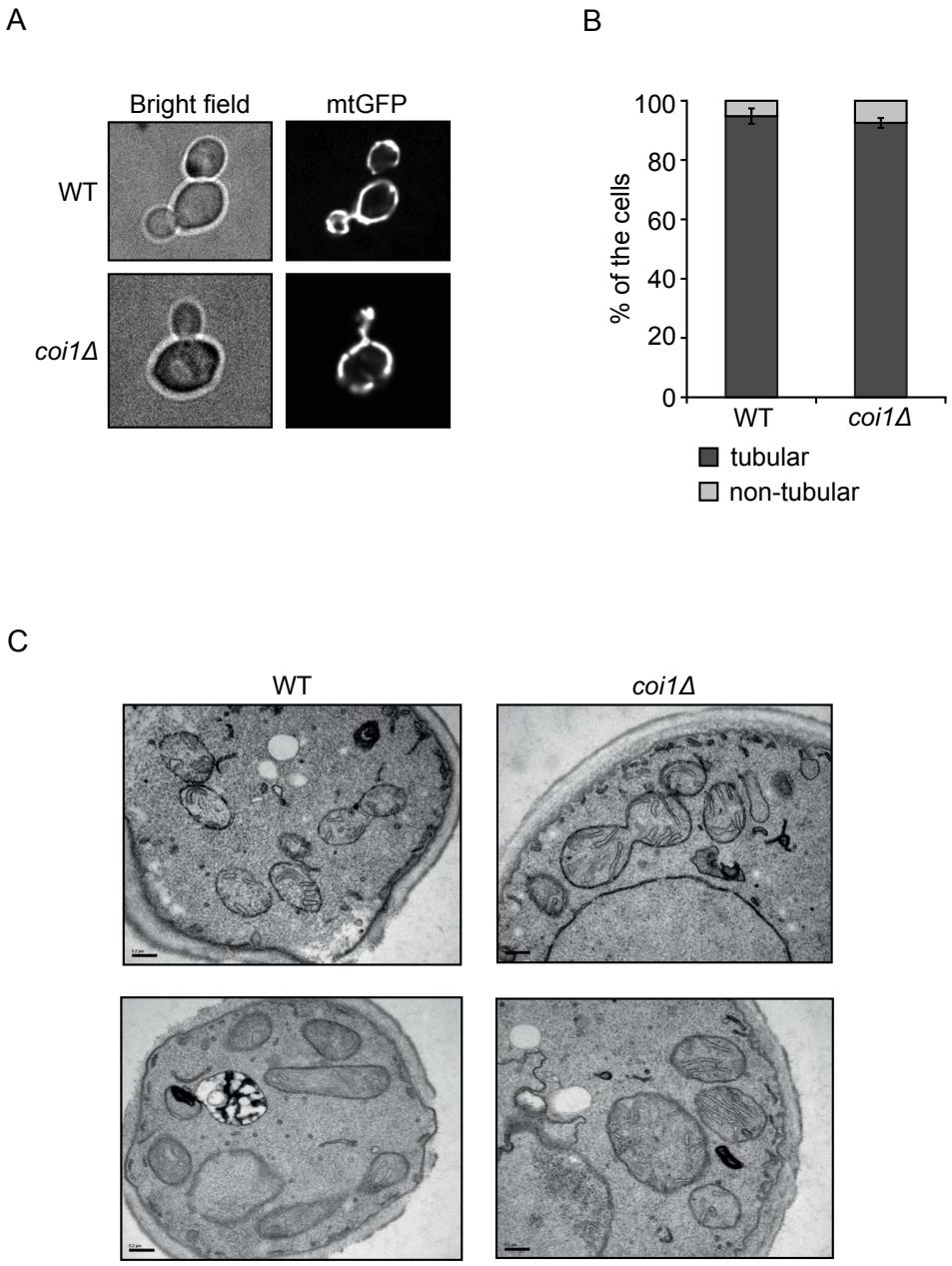


**Figure S4. Absence of Coi1 has no effect on mitochondria morphology and ultrastructure.** **(A)** Wild type and *coi1Δ* cells were transformed with plasmid expressing mitochondrially targeted GFP (mtGFP). The transformed cells were grown to logarithmic phase and then analysed by fluorescence microscopy. Representative cells of each strain are presented. **(B)** The quantification of both strains shows the average percentage with standard deviation bars of three independent experiments with at least 100 cells per experiment (n = 3). **(C)** Wild type and *coi1Δ* cells were grown in rich glycerol-contaning medium (YPG) and analysed by thin-section transmission electron microscopy. Representative images of cellular sections are shown. Scale bars, 0.2 µm.

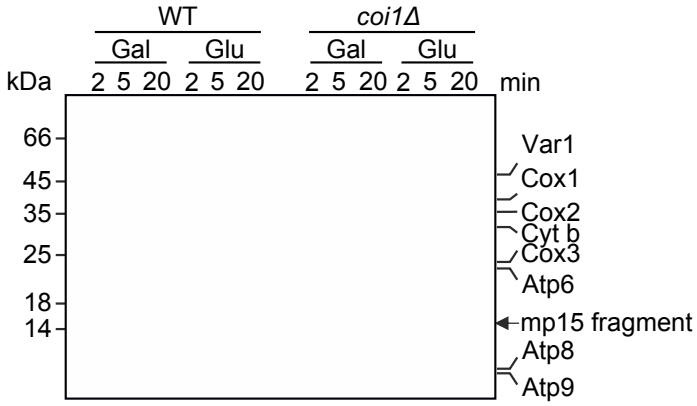


**Figure S5. Coi1 is not required for mitochondrial protein synthesis.** Wild type and *coi1Δ* cells were grown in rich media containing galactose (Gal) or glucose (Glu). Cells were re-suspended in synthetic medium lacking methionine and treated with cycloheximide to block cytosolic translation. Radiolabeled methionine was added and cells were incubated at 30°C. At the indicated time points samples were subjected to alkaline extraction and analysed by SDS-PAGE followed by autoradiography. The identities of the translated mitochondria proteins are indicated on the margin. MP15 is a proteolytic fragment of Cox1.

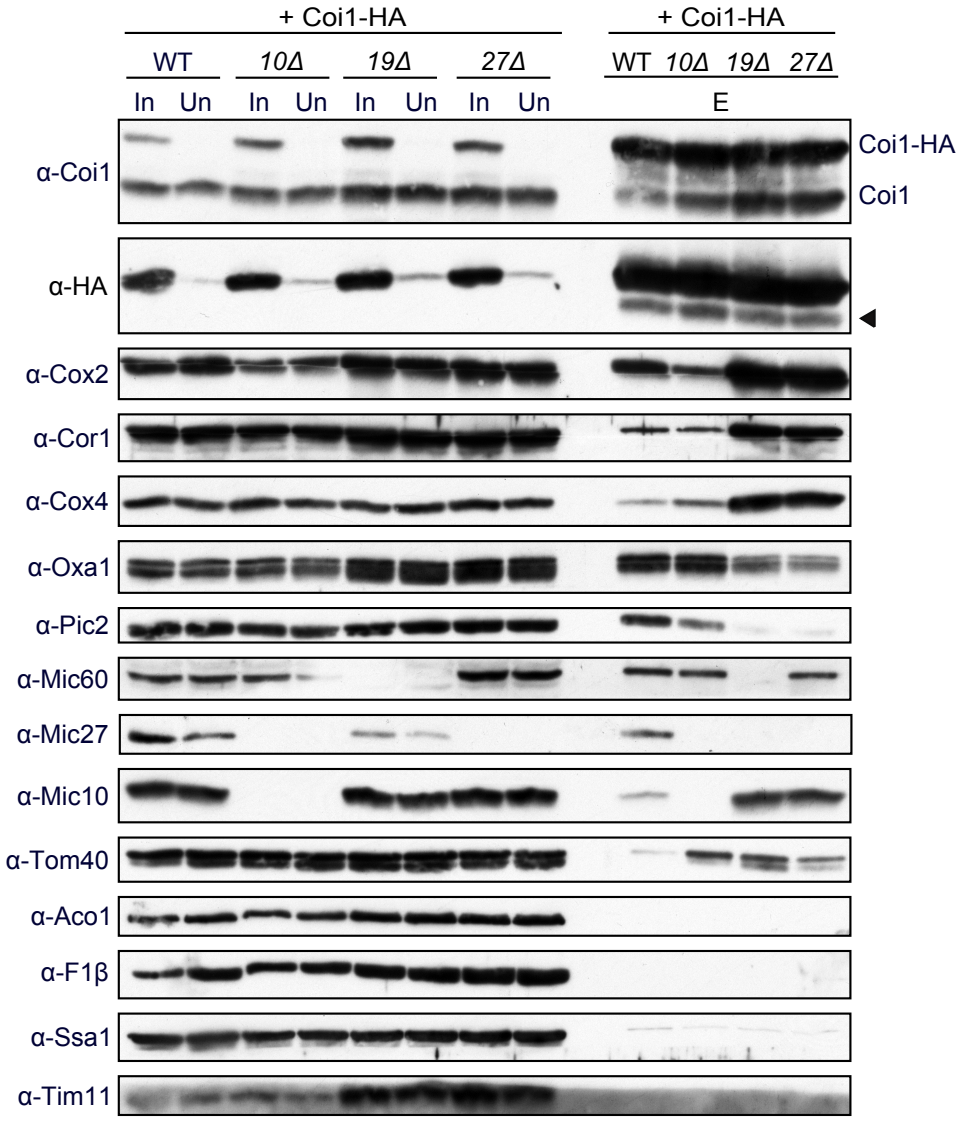


**Figure S6. The absence of MICOS subunits affects the interactions of Coi1 with various proteins.** Mitochondria were isolated from wild type, *mic10Δ* (10Δ), *mic19Δ* (19Δ), or *mic27Δ* (27Δ) cells expressing Coi1-HA. The organelles were lysed with buffer containing 0.5% Triton X-100 and after a clarifying spin the supernatants were subjected to pull-down with anti-HA magnetic beads. Supernatant (In, 2% of total), unbound material (Un, 2% of total) and the eluates (E, 33.3% of total) were analyzed by SDS-PAGE and immunodecoration with the indicated antibodies. A degradation product of Coi1-HA is indicated with an arrowhead

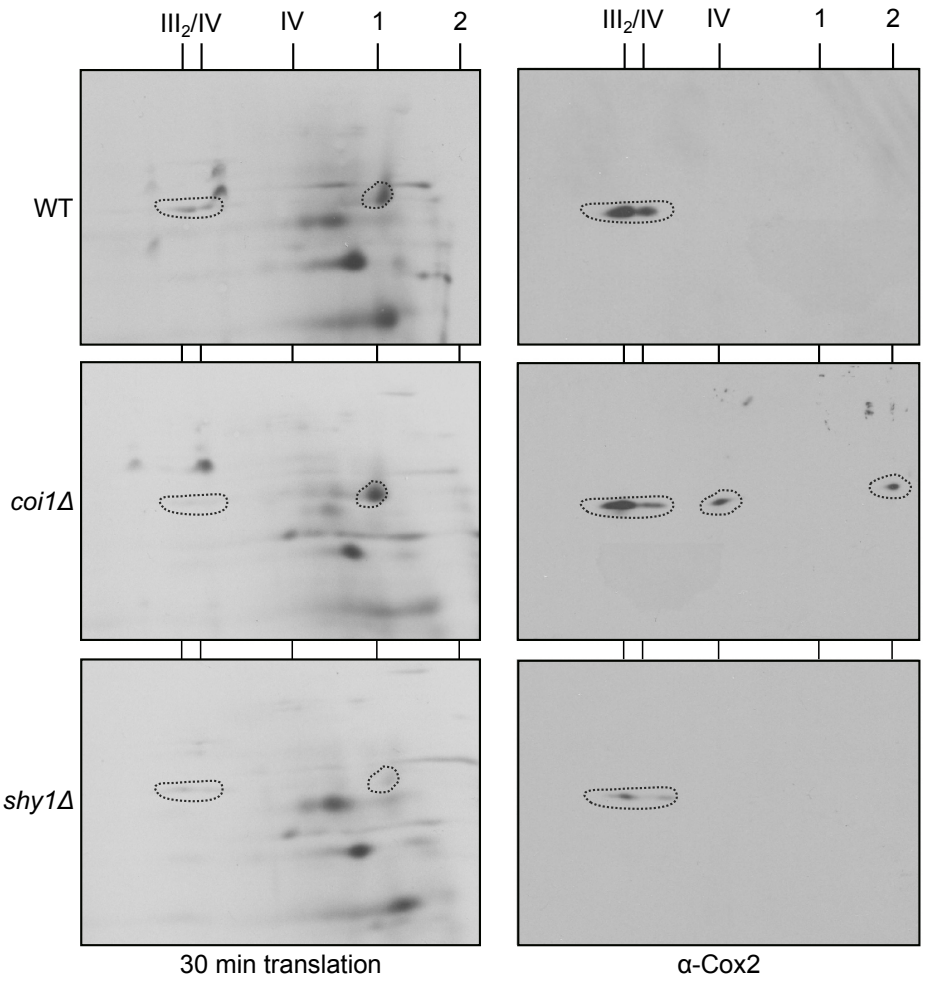


**Figure S7. Deletion of *COI1* results in compromised assembly of newly synthesized Cox2.** Translation products were radiolabeled in mitochondria isolated from wild type (WT), *coi1Δ* or *shy1Δ* cells. Mitochondria were then solubilized in digitonin-containing buffer and applied to native BN-PAGE (first dimension) followed by SDS-PAGE (second dimension). Proteins were transferred onto PDVF membranes and visualized by autoradiography. Postions of newly synthesized radiolabeled Cox2 are indicated by dotted lines. Right panels: The membranes shown on the left were used for immunodecoration with anti-Cox2 antibodies. Cox2 signals are indicated by dotted lines. Various Cox2-containing species are indicated at the top of the panels: $III_2$/IV, supercomplexes containing complexes III and IV; IV, complex IV; 1, an early assembly intermediate containing newly synthesized, unassembled Cox2; 2, a small Cox2-containing species, which presumably represents a breakdown product.

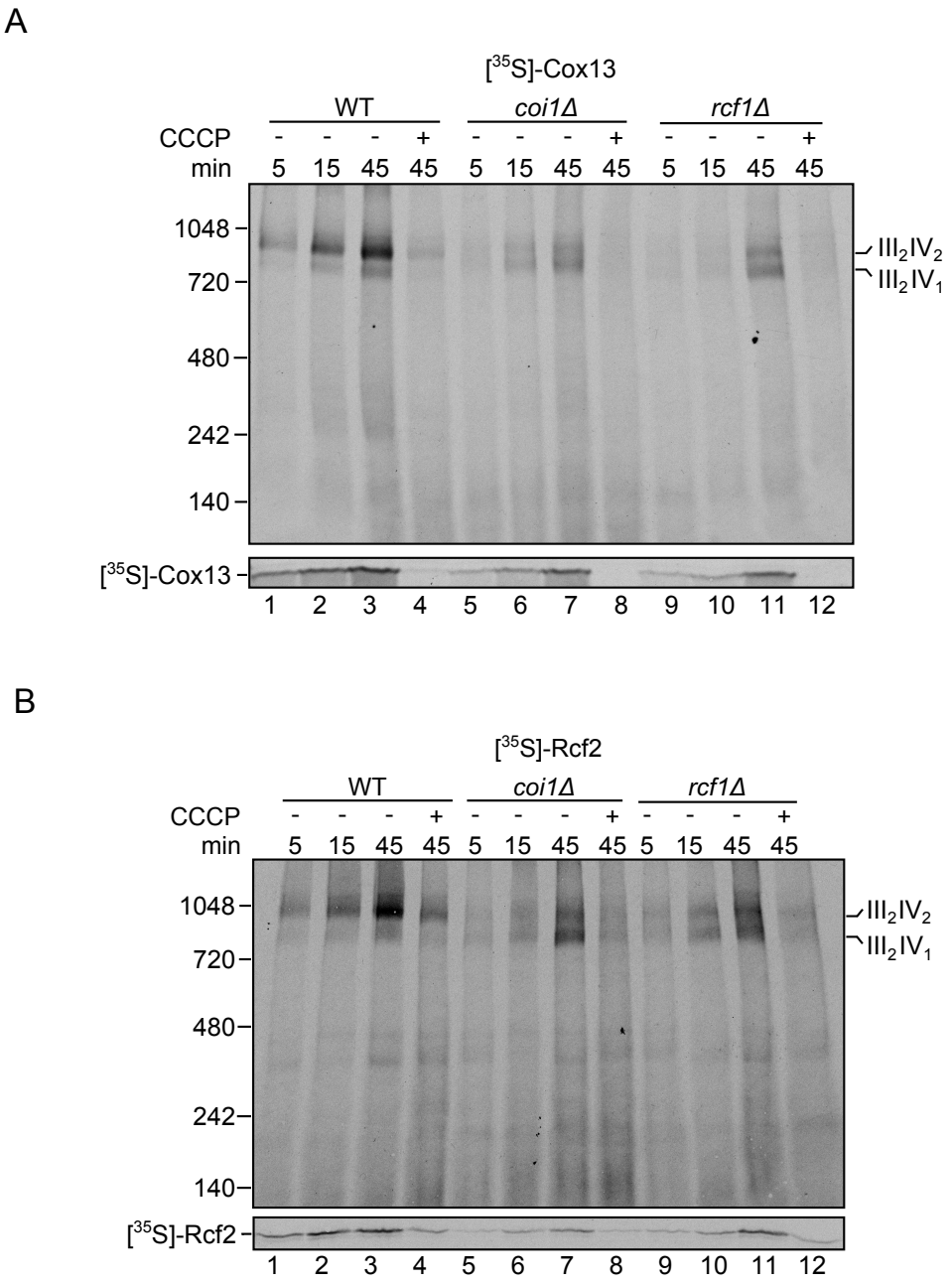


**Figure S8. Assembly of Cox13 and Rcf2 into supercomplexes is Coi1 dependent.** Radiolabeled precursors of Cox13 **(A)** and Rcf2 **(B)** were imported for the indicated time periods into mitochondria isolated from wild type, *coi1Δ* or *rcf1Δ* cells. In some samples CCCP was added to dissipate the ΔΨ. At the end of the import reactions, mitochondria were re-isolated and divided into two aliquots. One sample was solubilized with 1% digitonin and analysed by blue native-PAGE (upper panel). The other one was treated with Proteinase K to remove non-imported material and then analysed by SDS-PAGE (lower panel). Imported proteins were detected by autoradiography. The presented gels represent three independent experiments. The migration of the supercomplexes is indicated.

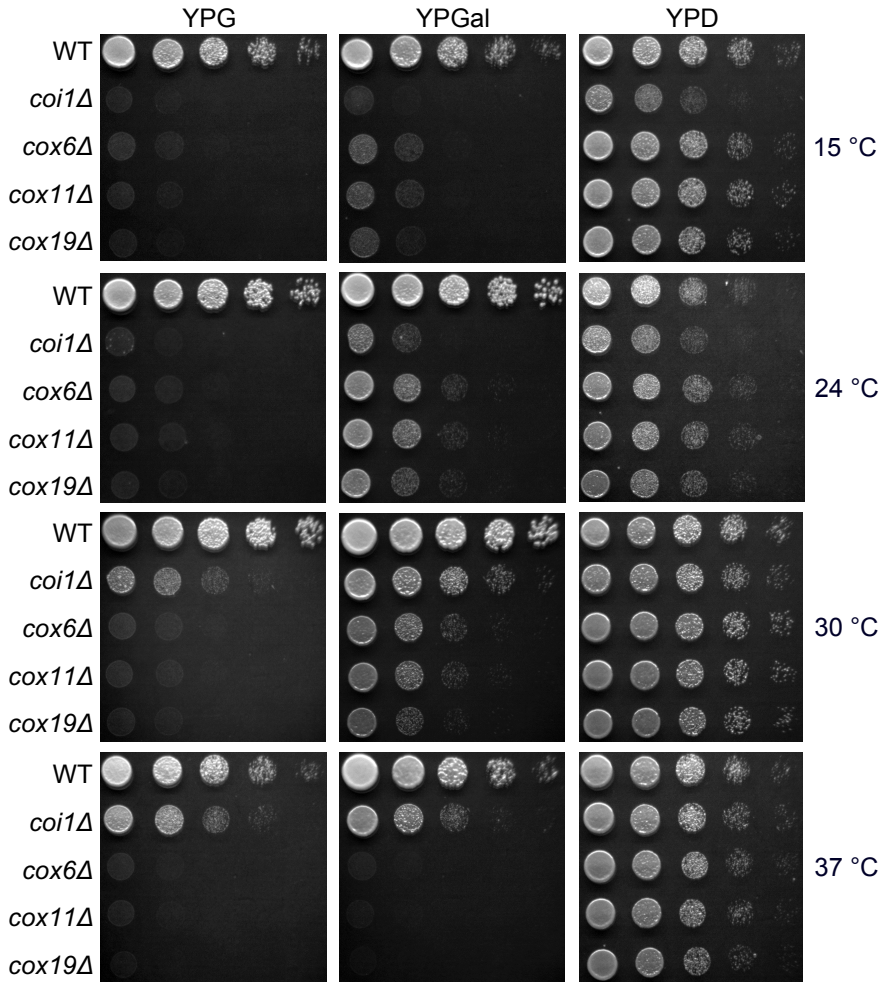


**Figure S9. The deletion of *COI1* results in a strong growth retardation at lower temperatures.** Wild type, *coi1Δ*, *cox6Δ*, *cox11Δ*, and *cox19Δ* cells were tested by drop dilution assay at the indicated temperatures on rich medium containing glycerol (YPG), galactose (YPGal), or glucose (YPD).

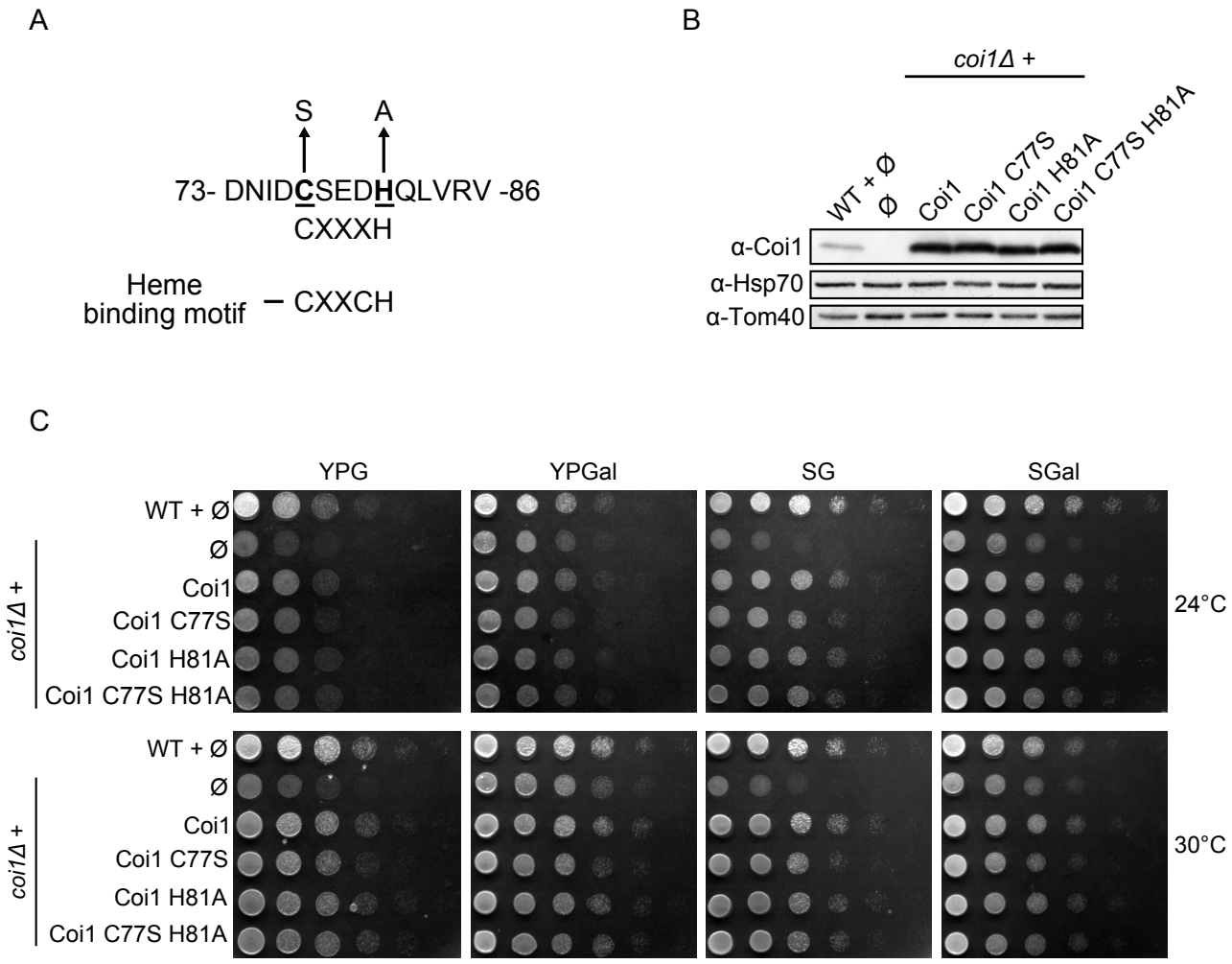


**Figure S10. CXXXH motif in Coi1 has no functional role. (A)** Schematic representation of the mutations preformed at the putative heme binding domain in Coi1. Cysteine 77 was mutated to serine (C77S) and histidine 81 to alanine (H81A). The sequence of the canonical heme binding motif is indicated. **(B)** Crude mitochondria was isolated from the indicated strains. Proteins were analyzed by SDS-PAGE and immuno-decorated against Coi1, Hsp70 (mitochondrial matrix) and Tom40 (OM protein). **(C)** Cells lacking Coi1 were transformed with an empty plasmid or plasmid encoding full length Coi1, single mutants (C77S and H81A), or double mutant (C77S/H81A). The growth on the specified medium of the transformed cells, and of WT cells as a control, was monitored at the indicated temperatures by drop-dilution assay.

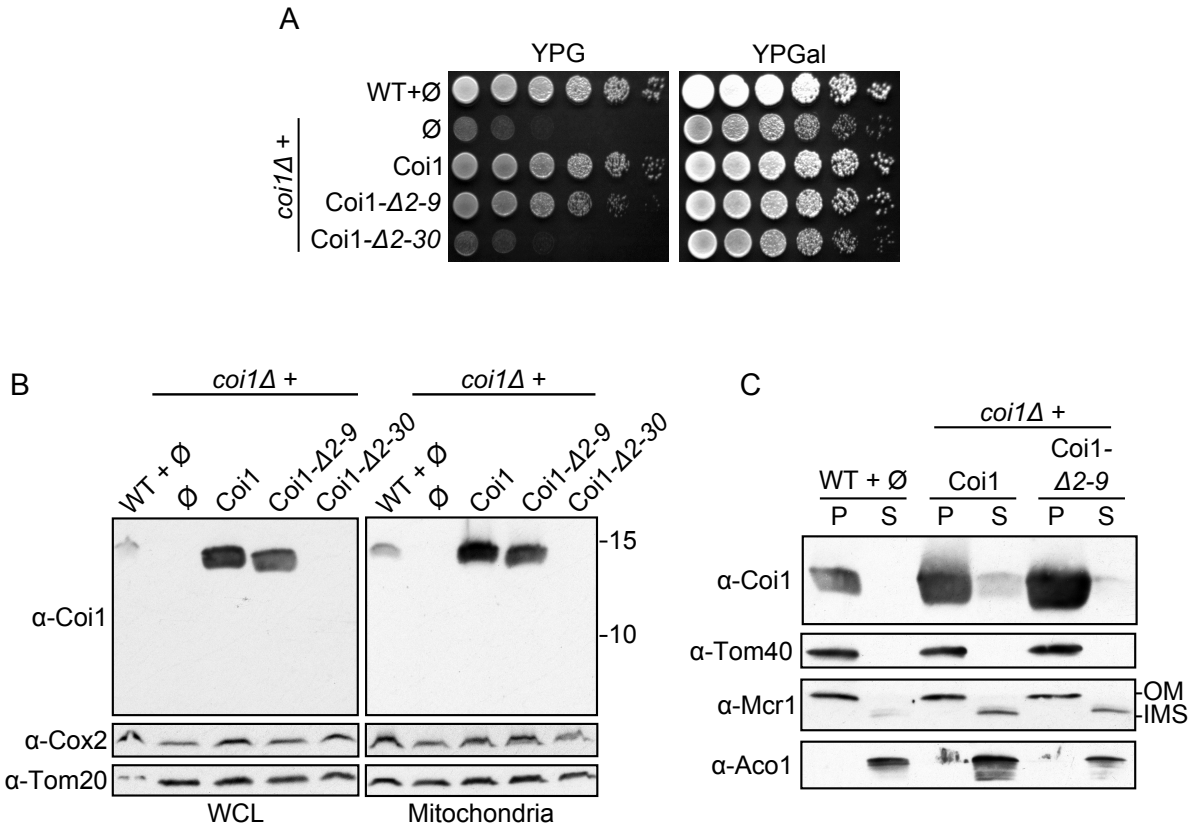


**Figure S11. The transmembrane segment of Coi1 is required for its function. (A)** *coi1Δ* cells were transformed with an empty plasmid or with a plasmid encoding either native Coi1 or the indicated Coi1 variant. The growth of the transformed cells on the specified medium, and of WT cells as a control, was monitored at 30°C by drop-dilution assay. **(B)** Crude mitochondria and whole cell lysate (WCL) were isolated from the indicated strains. Samples were analyzed by SDS-PAGE and immunodecoration with antibodies against Coi1, Cox2 (IM protein), and Tom20 (OM protein). **(C)** Isolated mitochondria (as described in part (B)) were subjected to alkaline extraction. The pellet (P) and the supernatant (S) fractions were analyzed by SDS-PAGE followed by immunodecoration with antibodies against the indicated proteins.

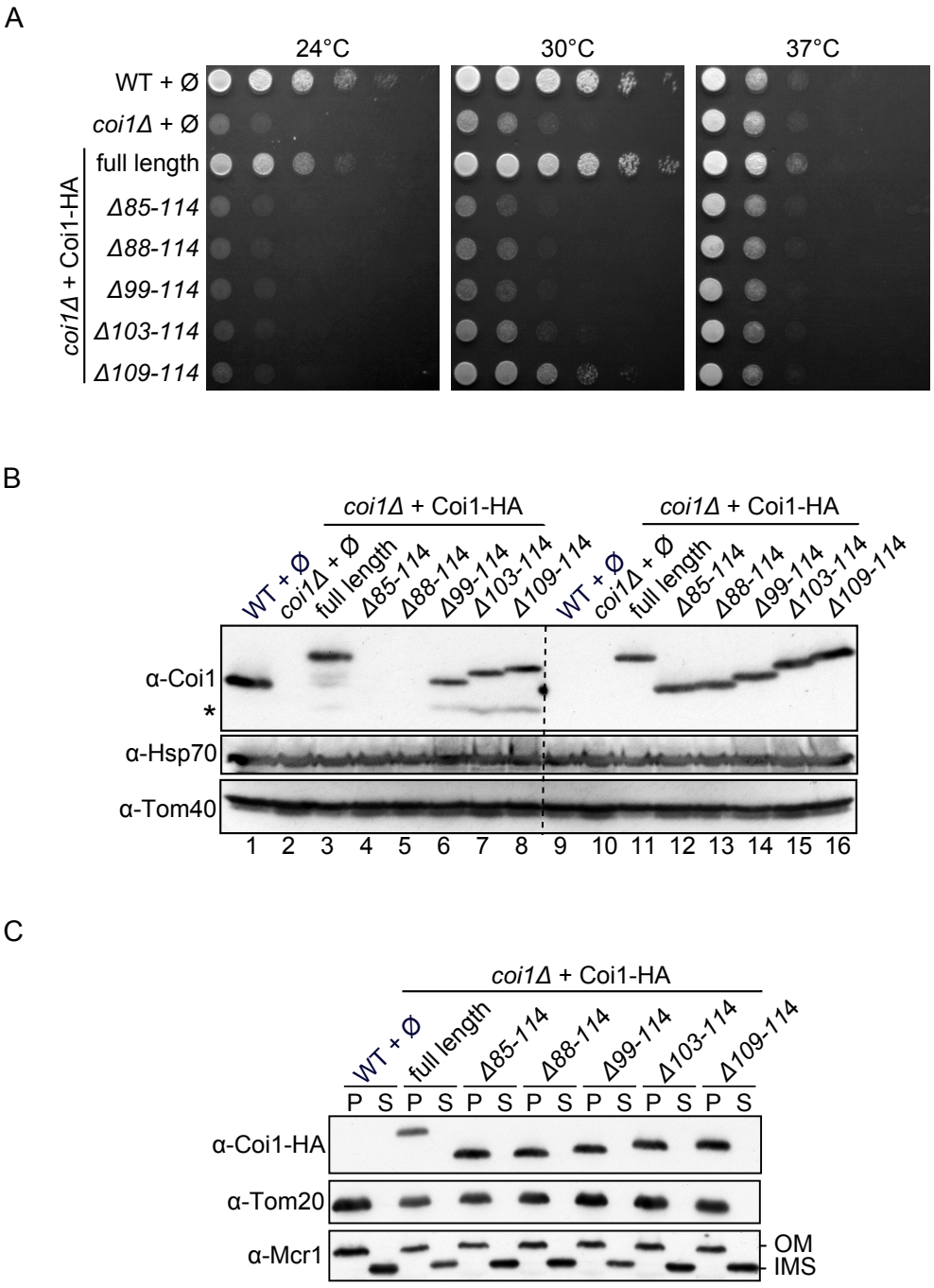


**Figure S12. Conserved residues at the C-terminal region of Coi1 are crucial for its function.**
**(A)** WT or *coi1Δ* cells were transformed with an empty plasmid or with a plasmid encoding the indicated HA-tagged variant of Coi1. The cells were subjected to a drop dilution assay at the indicated temperatures on selective glycerol-containing medium. **(B)** Isolated mitochondria (30 µg) from the indicated strains were analyzed by SDS-PAGE and immunodecoration with the specified antibodies. Lanes 1-8 were decorated with an antibody against Coi1 whereas lanes 9-16 with an antibody against the HA tag. *, a degradation product of Coi1. **(C)** Mitochondria isolated from the indicated strains were subjected to alkaline extraction. The pellet (P) and the supernatant (S) fractions were analyzed by SDS-PAGE followed by immunodecoration with antibodies against the indicated proteins. Antibodies against Coi1 were raised against a mixture of two peptides (peptide 1: a.a. 101-114 and peptide 2: a.a. 83-96).

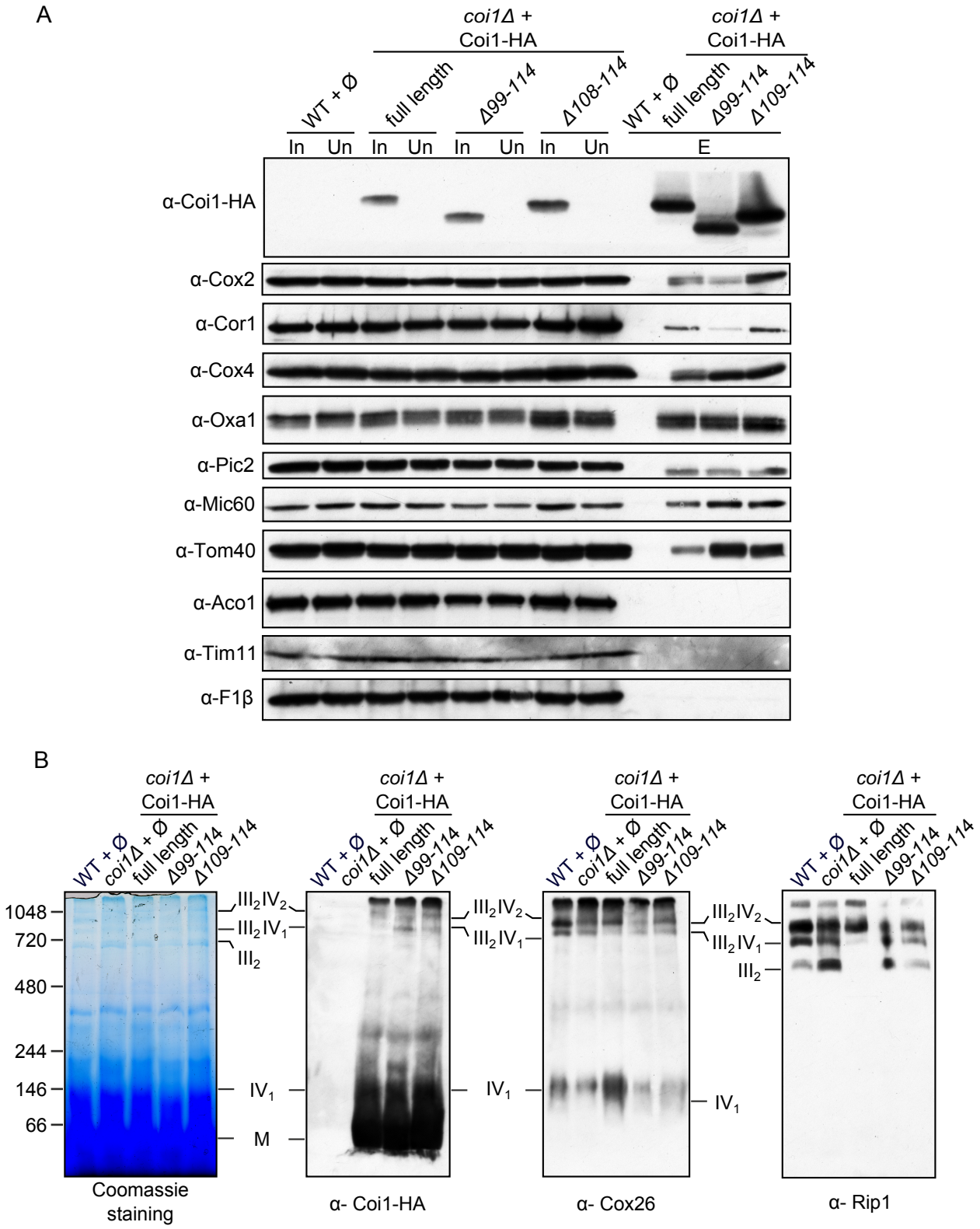


**Figure S13. Conserved residues at the C-terminal region of Coi1-HA are important for its function. (A)** Mitochondria were isolated from wild type and *coi1Δ* cells expressing the indicated HA-tagged variants of Coi1. Supernatant (In, 2% of total), unbound material (Un, 2% of total) and material eluted from the beads (E, 33.3% of total) were analyzed by SDS-PAGE and immunodecoration with the indicated antibodies. **(B)** Mitochondria isolated from the strains described in part (A) were analyzed by blue native PAGE. Samples were solubilized using 1% digitonin and the gels were stained with Coomassie, or immunodecorated with antibodies against the HA-tag (Coi1-HA), complex IV subunit Cox26, or the complex III component Rip1. M; Coi1-HA monomer.

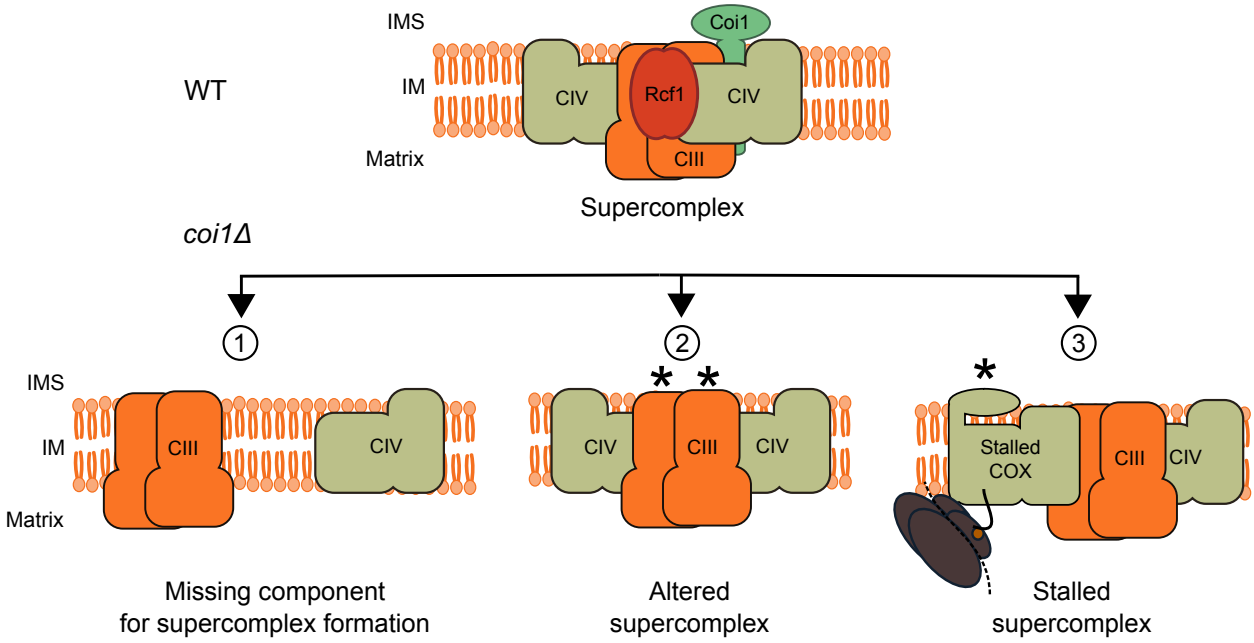


**Figure S14. Suggested models for the involvement of Coi1 in supercomplex formation.**
Upper part: In WT cells Coi1 and Rcf1 help to form supercomplexes of complex III and complex IV. Lower part: Deletion of *COI1* can lead to unassembled supercomplex and the coexistence of different pools such as **(1)** partially assembled supercomplexes, **(2)** altered structure of assembled supercomplexes, or **(3)** stalled assembly of complex IV due to defects in heme integration. *, stalled complex IV; **, altered supercomplex.