Supplemental material

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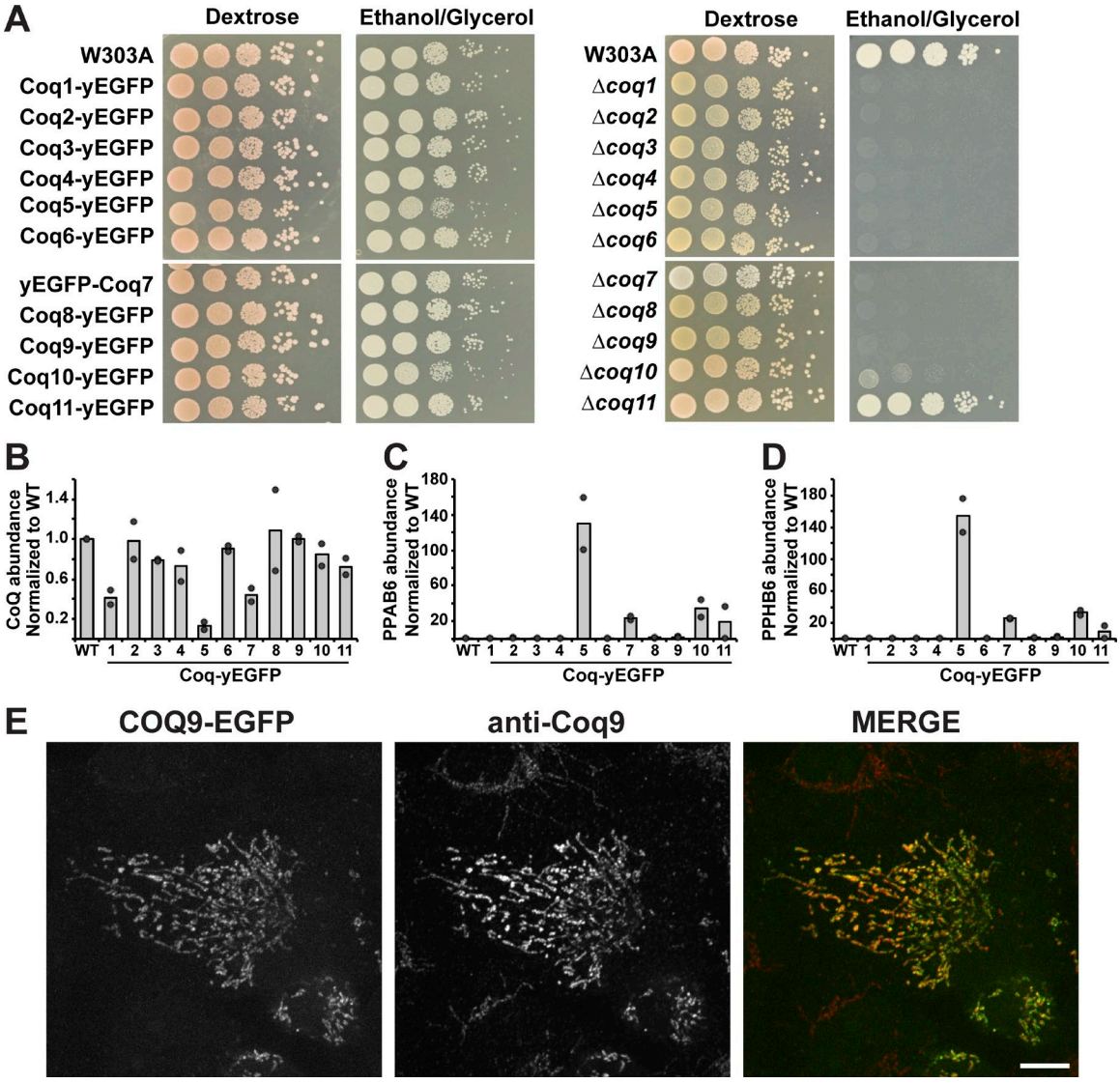

Figure S1. **Functional analysis of tagged Coq proteins in cells and cells lacking Coq proteins. (A)** Serial dilutions of indicated strains plated on media containing dextrose (left) and nonfermentable carbon source ethanol/glycerol (right). **(B–D)** Relative CoQ (B), PPAB6 (C), and PPHB6 (D) abundance measurements of indicated strains. Abundance measurements were normalized to WT. Bars represent mean of two independent experiments, and data points (circles) represent the measurements for each experiment. **(E)** Representative max z-projection image of fixed U2OS cells expressing pCOQ9-EGFP and labeled with anti-Coq9 antibody conjugated to Alexa Fluor 568 (red). Scale bars = 10 µm.

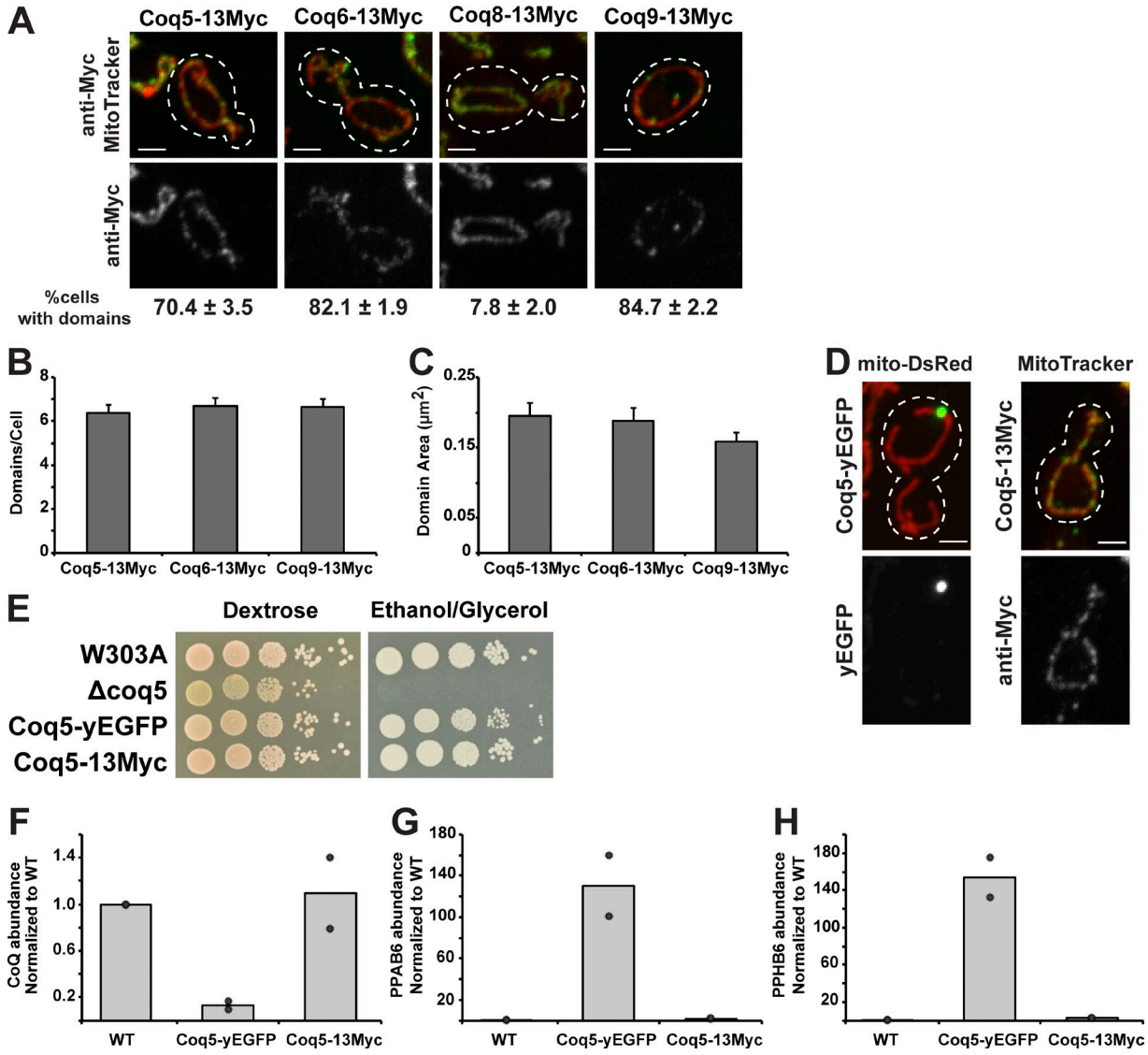

Figure S2. **Partially functional Coq5-yEGFP disrupts CoQ production and domain distribution. (A)** Representative max z-projection images of fixed yeast cells expressing Coq-13Myc from their endogenous loci labeled with anti-Myc antibody conjugated to Alexa Fluor 488 (green) and MitoTracker Red (red). Percentage of cells with CoQ domains corresponding to respective yeast strains are shown below images as mean ± SEM; *n* > 70 cells from three independent experiments. Dotted lines represent cell boundary. **(B and C)** Quantification of average number of domains per cell (B) and domain area in μm^2 (C) from cells imaged in A. Data represented as mean ± SEM; *n* > 70 cells from three independent experiments. Kruskal–Wallis test, P = 0.6 and 0.3, respectively, not significant. **(D)** Representative max z-projection images of cells expressing Coq5-yEGFP (green) and mito-DsRed (red) or Coq5-13Myc labeled with anti-Myc antibody conjugated to Alexa Fluor 488 (green) and MitoTracker Red (red). **(E)** Serial dilutions of indicated strains plated on media containing dextrose (left) and nonfermentable carbon source ethanol/glycerol (right). **(F–H)** Relative CoQ (F), PPAB6 (G), and PPHB6 (H) abundance measurements of indicated strains. Abundance measurements were normalized to WT. Bars represent mean of two independent experiments, and data points (circles) represent the measurements for each experiment. Scale bars = 2 μm.

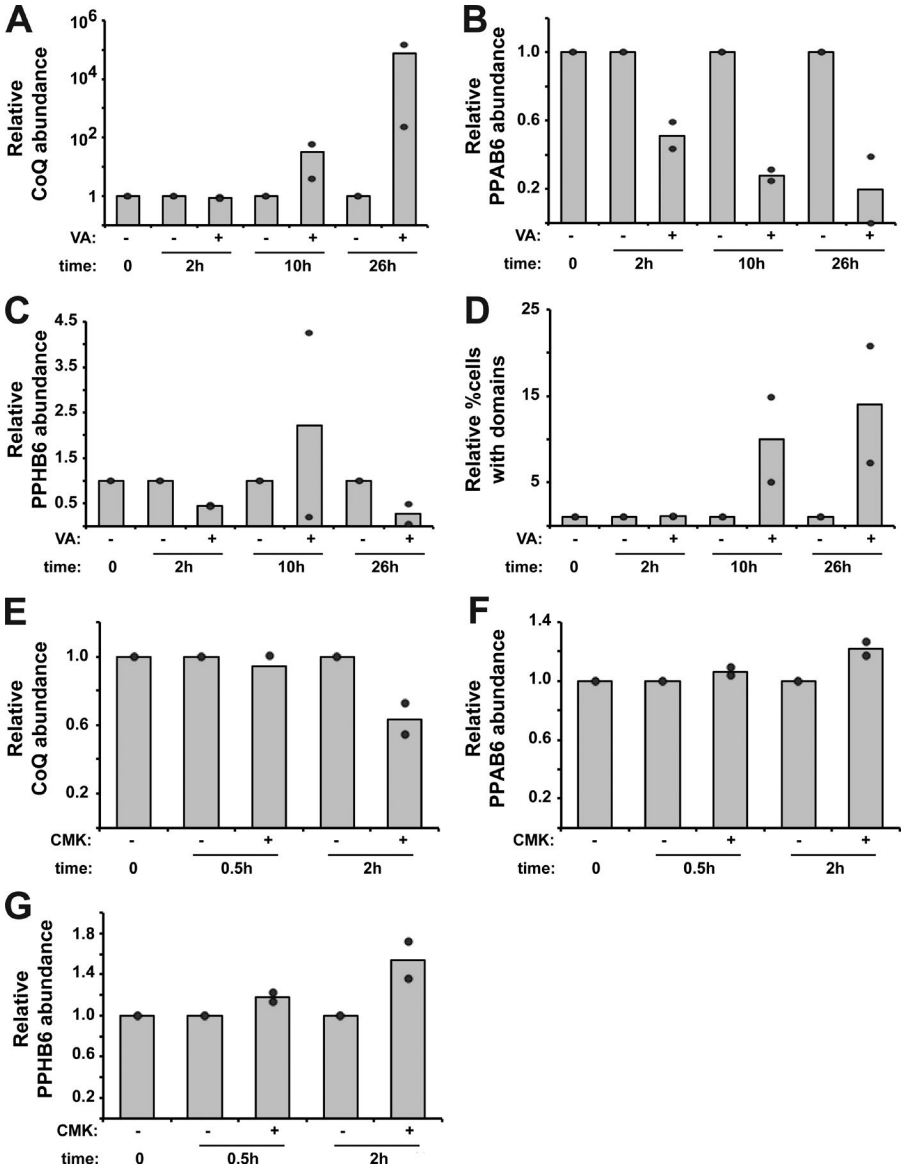

Figure S3. **CoQ domains are tightly correlated with CoQ production. (A–C)** Relative CoQ (A), PPAB6 (B), and PPHB6 (C) abundance measurements of Δ*coq6* yeast cells expressing Coq9-yEGFP from its endogenous loci, mito-DsRed, and Coq6 G386A N388D expressed from the *TRP* locus. Cells were grown in media without or with 1 mM VA for indicated times. Normalized abundance measurements to time 0 and to the corresponding –VA control for each experiment. Bars represent mean of two independent experiments, and data points (circles) represent the measurements for each experiment. **(D)** Relative percentage of cells with CoQ domains corresponding to respective yeast strains as shown in A–C at indicated fixed time points without and with VA. Percentage of cells with domains were normalized to time 0 and to the corresponding –VA control for each experiment. $n > 70$ cells from three independent experiments. **(E–G)** Relative CoQ (E), PPAB6 (F), and PPHB6 (G) abundance measurements of Δ*coq8* yeast cells expressing Coq9-yEGFP from its endogenous locus, mito-DsRed, and Coq8 V202C M303C (Coq8 AS) expressed from a 2-µm plasmid. Cells were grown to log phase in media without or with 1 mM CMK for indicated times. Normalized abundance measurements to time 0 and to the corresponding CMK control for each experiment. Bars represent mean of two independent experiments, and data points (circles) represent the individual measurement for each experiment.

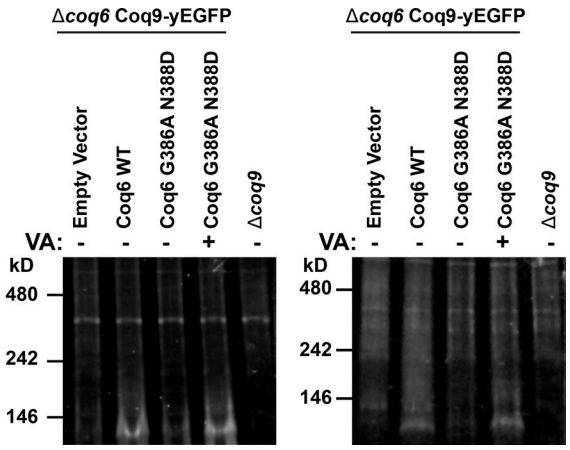

Figure S4. **Large molecular Coq9-containing species only in the presence of CoQ domains.** BN-PAGE analysis of isolated mitochondria from indicated strains immunoblotted using anti-yeast Coq9 antibodies. Δ*coq6* yeast cells expressing Coq9-yEGFP from its endogenous loci and empty vector, Coq6 WT, or Coq6 G386A N388D expressed from the *TRP* locus harvested for mitochondrial isolation experiments. Δ*coq6* yeast cells expressing Coq9-yEGFP and Coq6 G386A N388D were grown in media without or with VA for 26 h and harvested for mitochondrial isolation experiments. Δ*coq9* yeast cells were used a control. Arrow indicates Coq9-containing species. Data are shown from two independent experiments.

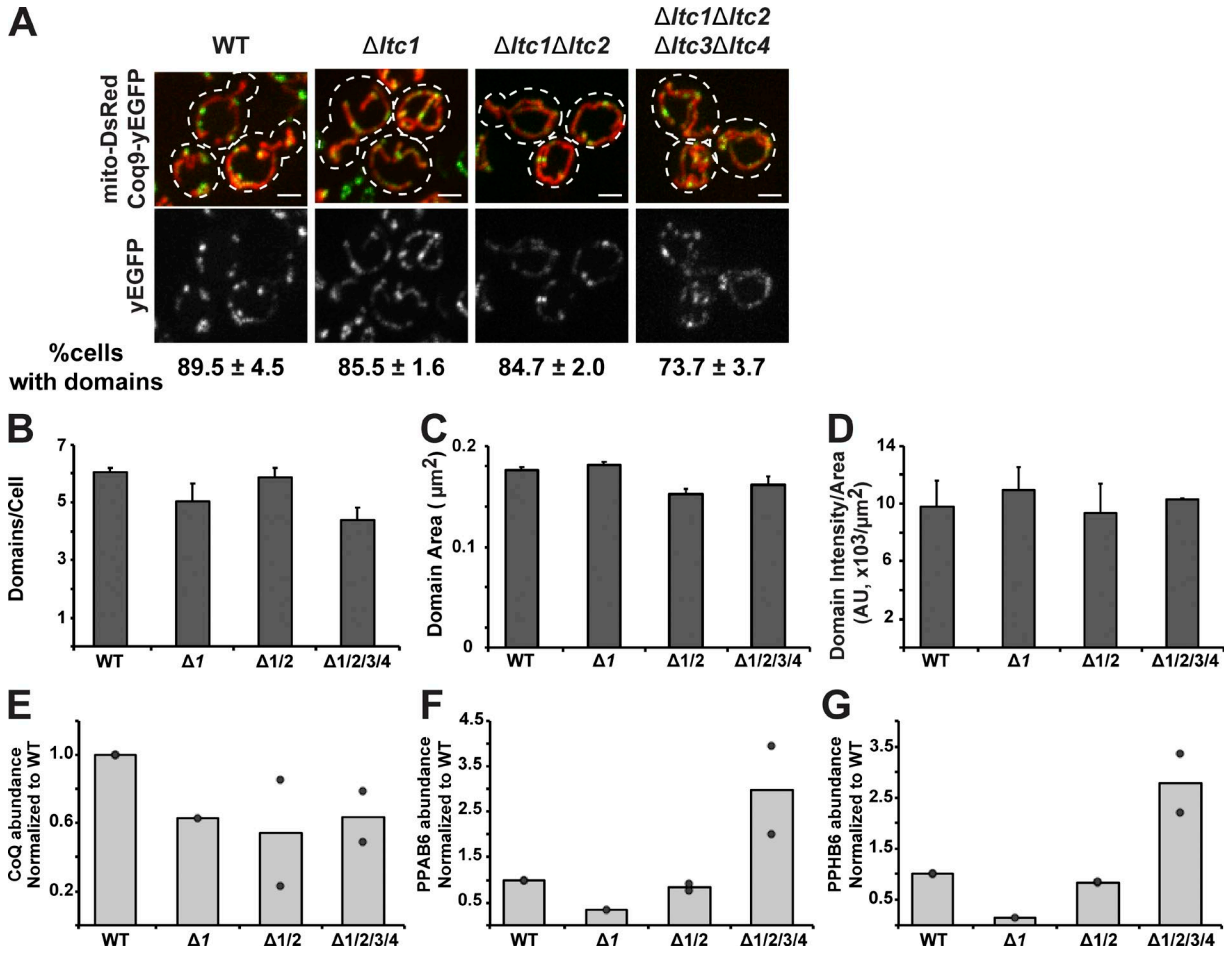

Figure S5. **CoQ domains are present in *LTC*-deficient cells. (A)** Representative maximum z-projection images of yeast cells expressing Coq9-yEGFP (green) from its endogenous loci and mito-DsRed (red) in indicated strains. Percentage of cells with CoQ domains corresponding to respective yeast strains are shown below images as mean ± SEM; $n > 70$ cells from three independent experiments. Dotted lines represent cell boundary. **(B–D)** Quantification of average number of domains per cell (B), domain area in μm^2 (C), and domain intensity per domain area in fluorescence intensity AU per μm^2 (D) from cells imaged in A. Data represented as mean ± SEM; $n > 70$ cells from three independent experiments. **(E–G)** Relative CoQ (E), PPAB6 (F), and PPHB6 (G) abundance measurements of indicated strains. Abundance measurements were normalized to the corresponding WT at each temperature. Bars represent mean of two independent experiments, and data points (circles) represent the measurements for each experiment. Scale bars = 2 μm.