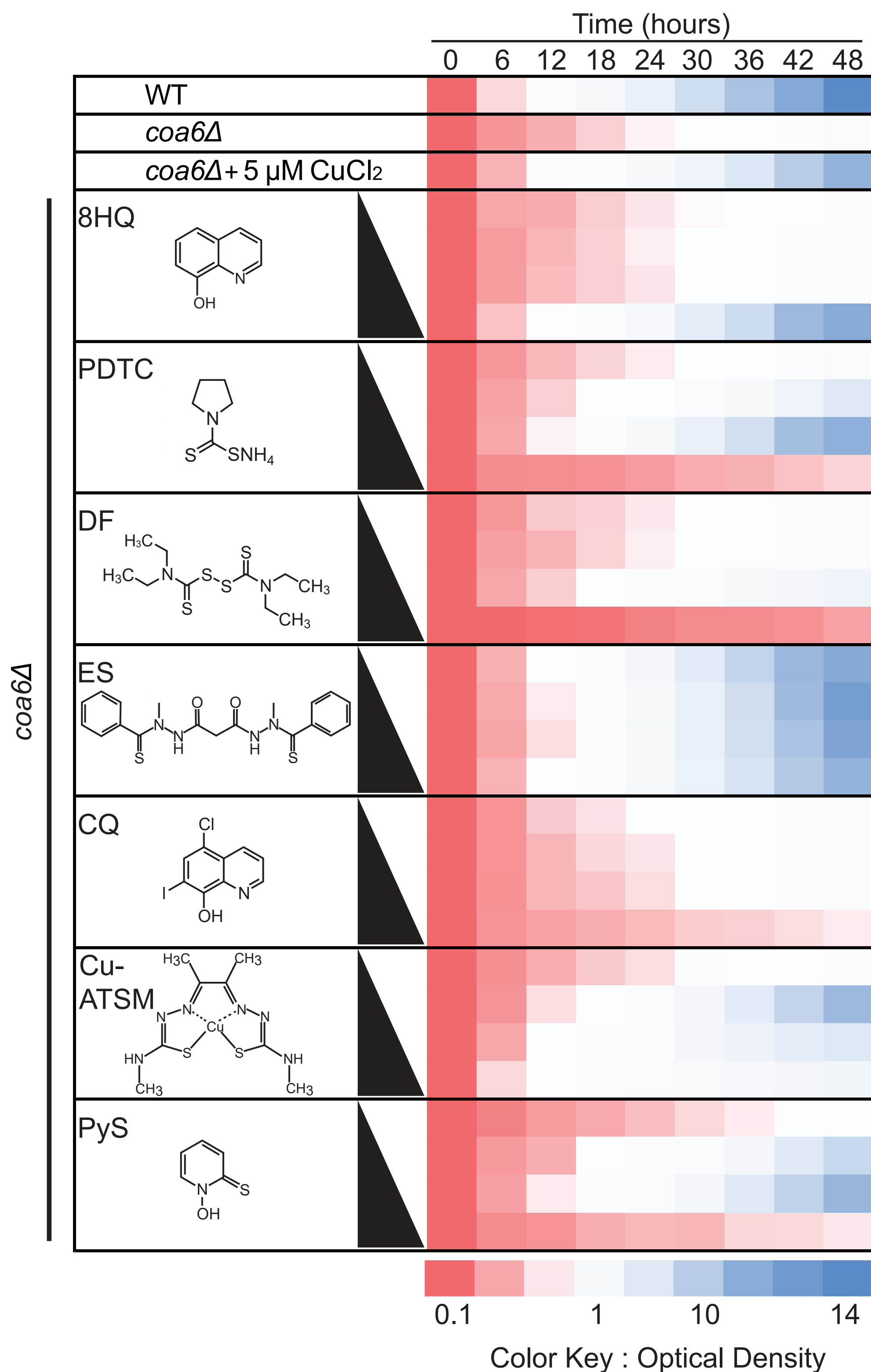


Fig. S1 A comparative analysis of copper binding agents identifies ES as the most potent pharmacological agent in rescuing respiratory growth deficiency of yeast *coa6Δ* cells. The respiratory growth of *coa6Δ* cells in YPGE supplemented with either 5μM copper chloride ($CuCl_2$) or with increasing concentrations (1nM to 1μM) of 8-hydroxyquinoline (8HQ), ammonium pyrrolidine dithiocarbamate (PDTC), disulfiram (DF), elesclomol (ES), clioquinol (CQ), diacetylbis(N(4)-methylthiosemicarbazonato) copper (II) (Cu-ATSM), and pyrithione (PyS) was measured at 37°C at the indicated time points. The data are representative of two independent measurements.

Supplementary Figure 1

www.pnas.org/cgi/doi/10.1073/pnas.1806296115

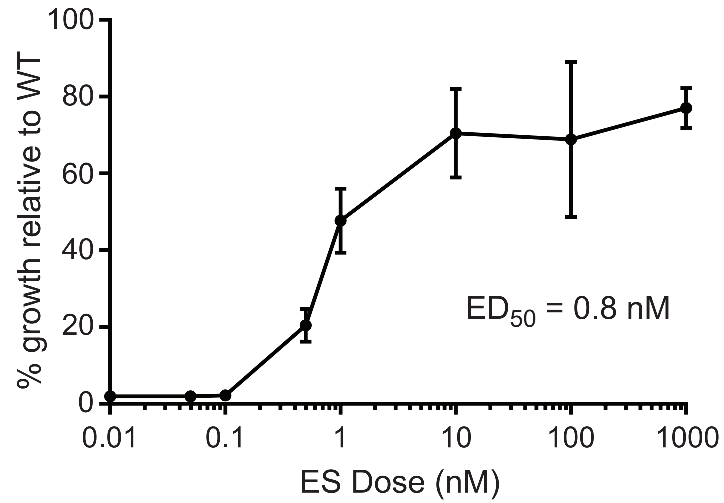


Fig. S2 Determination of the median effective dose (ED_{50}) of elesclomol (ES). Yeast *coa6Δ* cells were cultured in YPGE medium at 37°C in the presence of increasing concentrations (0.01nM to 1μM) of ES. The cell density was measured spectrophotometrically after 58 hours of growth at 600nm. The data represent the average ± SD from three independent measurements.

Supplementary Figure 2

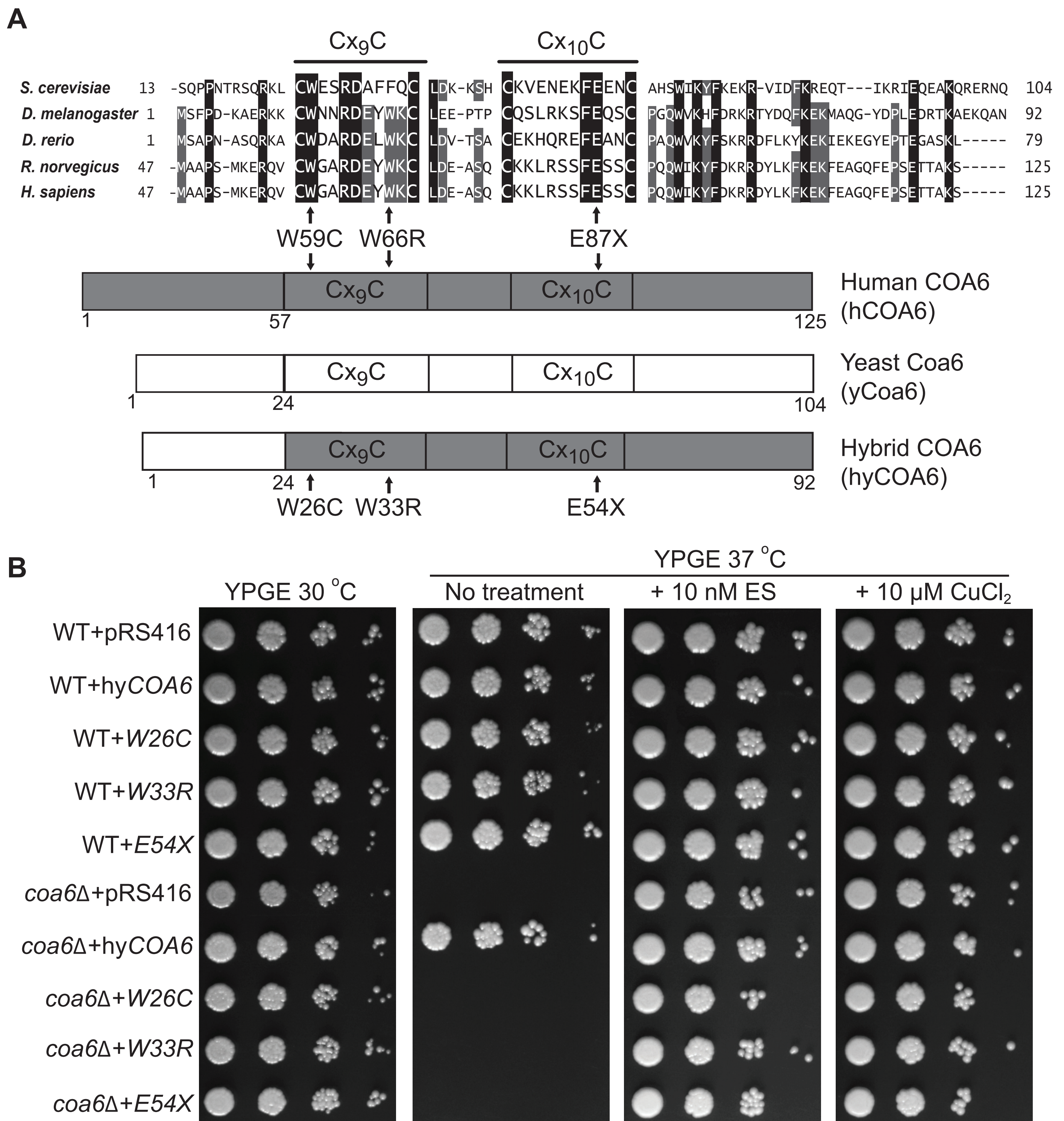


Fig. S3 ES supplementation restores respiratory growth of yeast cells expressing pathogenic *COA6* variants. (A) Sequence alignment of the conserved region of COA6 across indicated model organisms. Horizontal lines above Cx_9C and $Cx_{10}C$ residues show the conserved $Cx_9Cx_nCx_{10}C$ motif. Schematic representation of yeast-human hybrid (hyCOA6) protein, where the sequence from yeast Coa6 is shown in white, and the sequence from human COA6 is shown in gray. Arrows indicate amino acid residues that were mutated in human COA6 patients and the corresponding residues in the hyCOA6 protein. (B) WT and *coa6Δ* cells transformed with pRS416 empty vector or pRS416 vector expressing either hyCOA6 or hyCOA6 harboring patient mutations (W26C, W33R and E54X) were serially diluted and seeded on YPGE plates alone or those supplemented with either 10 nM ES or 10 μM copper chloride. Images were taken after 3 days for cells grown at 30°C and 5 days for cells grown at 37°C.

Supplementary Figure 3

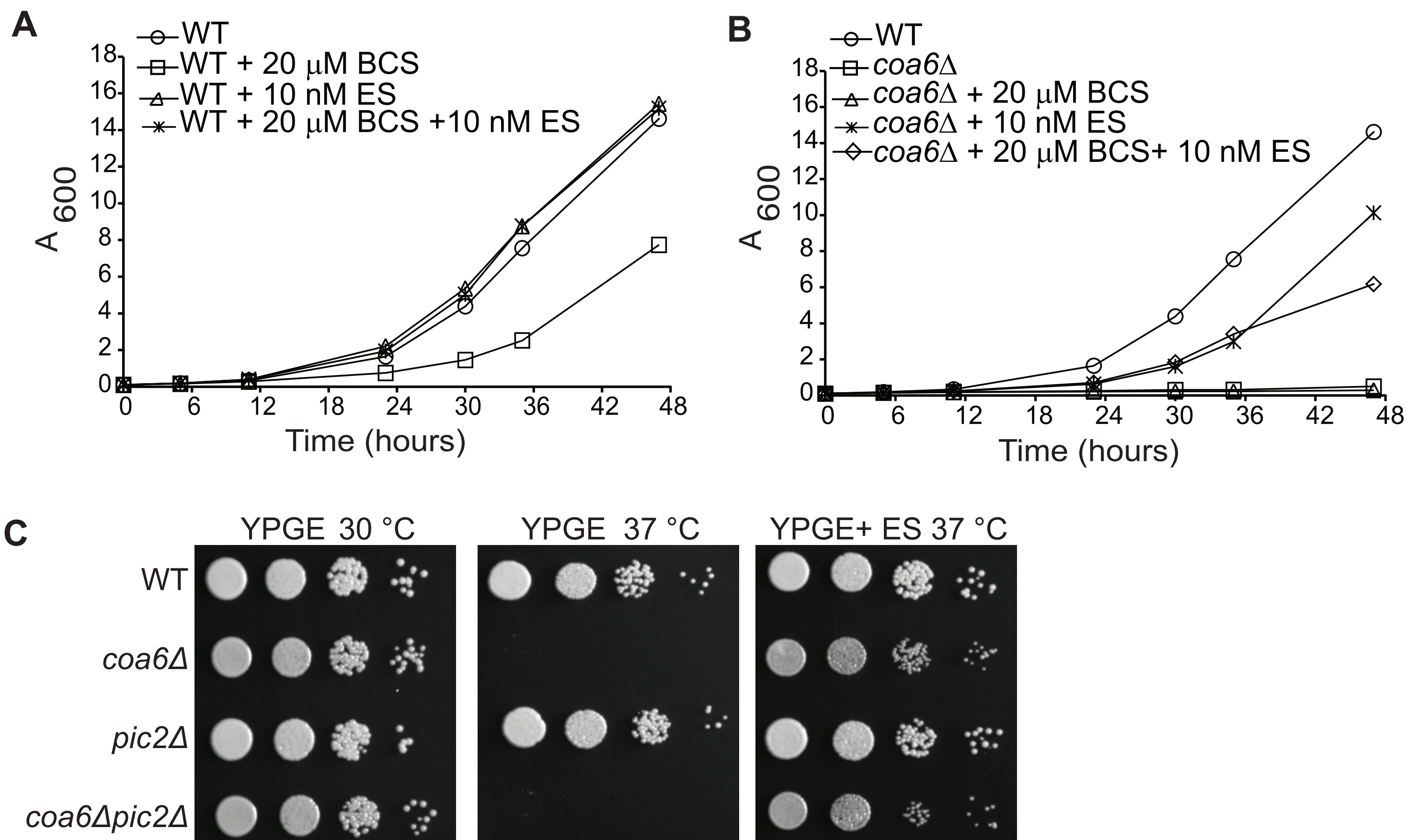


Fig. S4 Elesclomol (ES) rescue of respiratory growth of *coa6Δ* cells is dependent on copper availability but is independent of Pic2, a putative mitochondrial copper transporter. (A) BY4741 wild type (WT) and (B) *coa6Δ* cells were cultured in YPGE medium at 37 °C in the presence of 10nM ES, 20μM of the copper chelator bathocuproine disulfonic acid (BCS), or a combination of both. The cell density was measured spectrophotometrically at the indicated time points at 600nm. The data are representative of two independent experiments. (C) Serially diluted WT, *coa6Δ, pic2Δ,* and *coa6Δpic2Δ* double mutants were seeded on YPGE plates with and without 10nM ES supplementation and incubated at the indicated temperatures for 4 days before imaging. The data are representative of two independent experiments.

Supplementary Figure 4

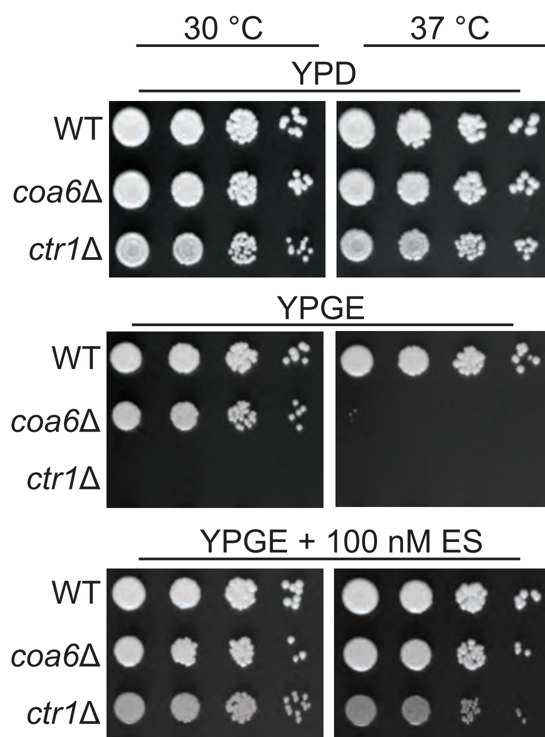


Fig. S5 Respiratory growth rescue of *ctr1Δ* cells is more pronounced with 100 nM elesclomol supplementation. Serially diluted WT, *coa6Δ,* and *ctr1Δ* cells were cultured under the indicated growth conditions for 4 days before imaging. The data are representative of three independent experiments.

Supplementary Figure 5

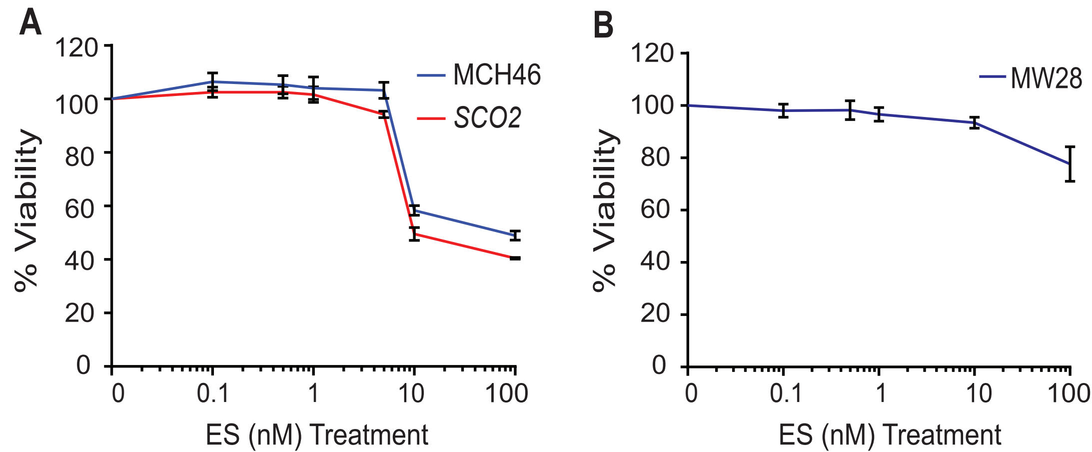


Fig. S6 Cell viability of immortalized and primary human fibroblasts cell lines treated with increasing concentrations of elesclomol (ES). (A) Immortalized control (MCH46) and *SCO2* patient fibroblasts and (B) primary human skin fibroblasts (MW28) cultured in the DMEM glucose – containing media were treated with increasing concentrations of ES for 36 hours. Cell viability was measured by quantifying total cellular ATP levels by Cell-Titre GLo assay (Promega). Data are expressed as mean ± SD (n=3).

Supplementary Figure 6

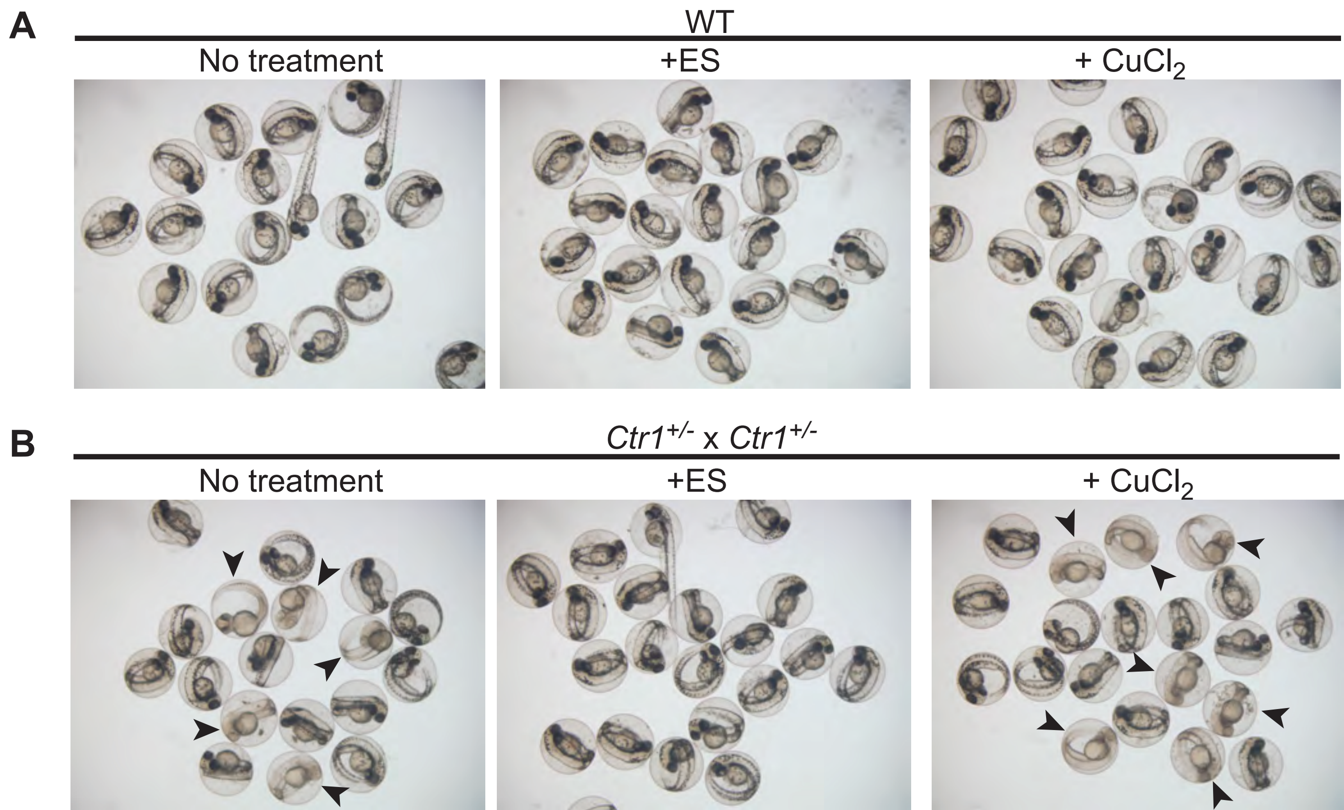
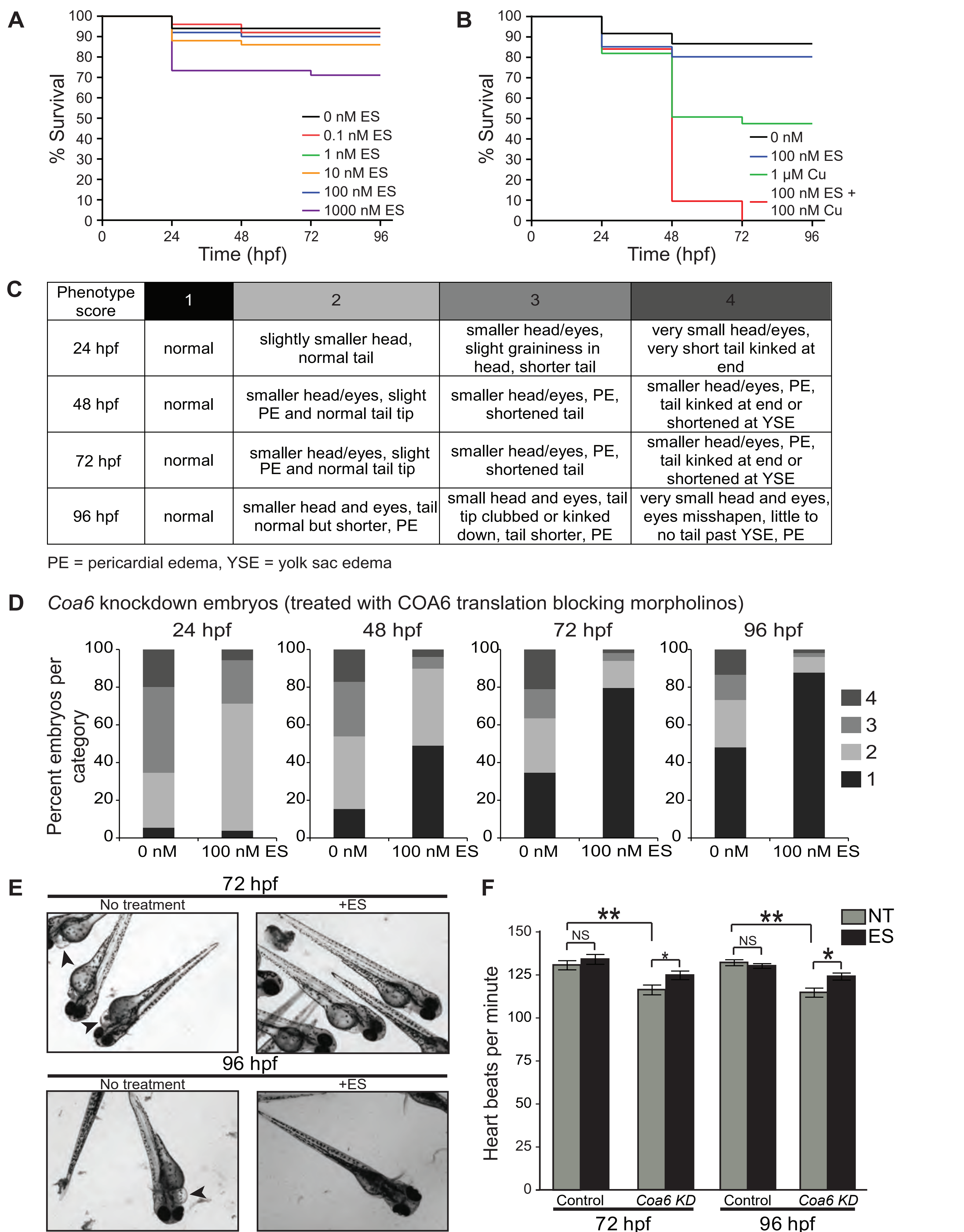


Fig. S7 ES supplementation rescues pigmentation of *Ctr1* knockout zebrafish while equivalent concentration of copper fails to rescue this defect. (A) Clutches of embryos from a WT zebrafish and (B) from a cross of a pair of *Ctr1*$^{+/-}$ heterozygous zebrafish were imaged at 48hpf following treatment with and without 100 nM elesclomol (ES) or 100 nM copper chloride ($CuCl_2$). Arrowheads in (B) indicate homozygous *Ctr1*$^{-/-}$ embryos with a pigmentation defect. Notably, not a single embryo with pigmentation defect was observed upon ES treatment.

Supplementary Figure 7



Phenotype score	1	2	3	4
24 hpf	normal	slightly smaller head, normal tail	smaller head/eyes, slight graininess in head, shorter tail	very small head/eyes, very short tail kinked at end
48 hpf	normal	smaller head/eyes, slight PE and normal tail tip	smaller head/eyes, PE, shortened tail	smaller head/eyes, PE, tail kinked at end or shortened at YSE
72 hpf	normal	smaller head/eyes, slight PE and normal tail tip	smaller head/eyes, PE, shortened tail	smaller head/eyes, PE, tail kinked at end or shortened at YSE
96 hpf	normal	smaller head and eyes, tail normal but shorter, PE	small head and eyes, tail tip clubbed or kinked down, tail shorter, PE	very small head and eyes, eyes misshapen, little to no tail past YSE, PE

Fig. S8 ES treatment improves morphological defects observed in *Coa6* knockdown embryos. (A) Zebrafish embryos were treated with indicated concentrations of ES and (B) copper, and combination of both at 3 hours post fertilization (hpf). The surviving fish were counted at the indicated time points. (n ≥30 for each treatment). (C) Table describing phenotype scores (1-4) at different time points in zebrafish embryo development. (D) Phenotypic scores of untreated and ES treated *Coa6* knockdown zebrafish embryos at the indicated time points (hpf, hours post fertilization). Batches of 50 fertilized embryos injected with either translation blocking or mismatch control morpholinos were treated with or without 100 nM ES at 3 hpf, before making observations at the indicated time points. (n≥30 for each time point and treatment). (E) Representative images of untreated and ES treated *Coa6* knockdown zebrafish embryos at 72 and 96 hpf. The arrow heads indicates cardiac edema. (F) Heart rate of zebrafish embryos treated with either mismatch control morpholino or the *Coa6* translation blocking morpholino were measured at the indicated time point. (n≥30 per group; data represent mean ± SEM).

Name	Sequence (5’ ➔ 3´)
Site Directed mutagenesis primers	
Hy*COA6* W26C Forward	agtattcatctctagcaccgcaacacaattttctttggg
Hy*COA6* W26C Reverse	cccaaagaaaattgtgttgcggtgctagagatgaatact
Hy*COA6* W33R Forward	ttttcgtccaaacatttccggtattcatctctagcaccc
Hy*COA6* W33R Reverse	gggtgctagagatgaataccggaaatgtttggacgaaaa
Hy*COA6* E54X Forward	cattgttgtggacaagaagattagaaagaggatctcaacttctta
Hy*COA6* E54X Reverse	taagaagttgagatcctctttctaatcttcttgtccacaacaatg

Fig. S9 Primers used in this study.

Supplementary Figure 9

Strain	Genotype	Source
BY4741 WT	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*	Greenberg lab
BY4741 *coa6Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *coa6*Δ:: *KanMX4*	Open Biosystems
BY4741 *sco1Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *sco1*Δ:: *KanMX4*	Open Biosystems
BY4741 *sco2Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *sco2*Δ:: *KanMX4*	Open Biosystems
BY4741 *cox12Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *cox12*Δ:: *KanMX4*	Open Biosystems
BY4741 *ctr1Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *ctr1*Δ:: *KanMX4*	Open Biosystems
BY4741 *atx1Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *atx1*Δ:: *KanMX4*	Open Biosystems
BY4741 *ccs1Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *ccs1*Δ:: *KanMX4*	Open Biosystems
BY4741 *gsh1Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *gsh1*Δ:: *KanMX4*	Open Biosystems
BY4741 *ccc2Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *ccc2*Δ:: *KanMX4*	Open Biosystems
BY4741 *gef1Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *gef1*Δ:: *KanMX4*	Open Biosystems
BY4741 *pic2Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *pic2*Δ:: *KanMX4*	Open Biosystems
STY11 *pic2Δcoa6Δ*	MAT a, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *pic2*Δ::*KanMX4*,*coa6*Δ::*NatMX4*	Ref (10)

Fig. S10 Yeast strains used in this study

Supplementary Figure 10