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Supplementary Data

The Metal Chelating and Chaperoning Effects of Clioquinol: Insights from Yeast Studies

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Table S1
Saccharomyces cerevisiae strains used in this work

Strain	Genotype	Source
BY4742	*MATα his3 leu2 lys2 ura3*	Invitrogen
Δ*ctr1*	*MATα his3 leu2 lys2 ura3 ctr1*::*KanMX4*	Invitrogen
Δ*fre1* Δ*fre2*	*MATα his3 leu2 lys2 ura3 fre1*::*KanMX4 fre2*::*URA3*	This work
Δ*mac1*	*MATα his3 leu2 lys2 ura3 mac1*::*URA3*	This work
DY1457	*MATα ade6 canl his3 leu2 trpl ura3*	David J. Eide
ZHY3	*MATα ade6 canl his3 leu2 trpl ura3 zrtl::LEU2 zrt2::HIS3*	David J. Eide
FO1	*MATa his3 leu2 lys2 ura3 OM45*::*GFP-HIS3* YEp352-*FUR4-GFP*	This work
OG45	*MATa his3 leu2 lys2 ura3 OM45*::*GFP-HIS3*	Li Yu
GG1	*MATa his3 leu2 lys2 ura3 GTR1*::*GFP-HIS3*	Li Yu
AG2	*MATα his3 leu2 lys2 ura3 ACO1*::*GFP-HIS3*	This work
PG8	*MATα his3 leu2 lys2 ura3 PHO8*::*GFP-HIS3*	This work

Table S2
Primers for RT-PCR

Gene	Forward primer (5'to3')	Reverse primer (5'to3')
ACT1	CCTACGTTGGTGATGAAGCT	GTCAGTCAAATCTCTACCGG
FRE5	GGACAAAGCACGCGAACACATT	GCCCTCTGACCCGCAACAAGT
FRE7	ACTCGCCGACTACCCCTTCTG	ATGCCTTCCACCGTTGATGC
VEL1	TGCAACAACCGTCAGATT	TGGGTAACAGGAAGCAACT

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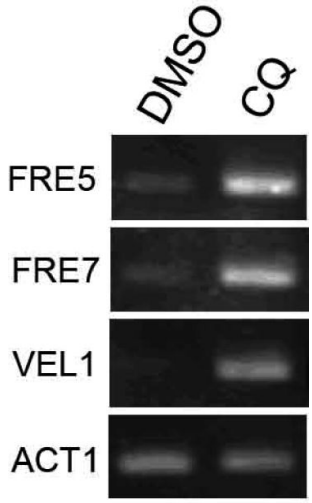

Figure S1. RT-PCR verification of some metal genes of interest. Transcription of *FRE5*, *FRE7*, and *VEL1* are upregulated by CQ treatment, while the housekeeping gene *ACT1* is not changed.

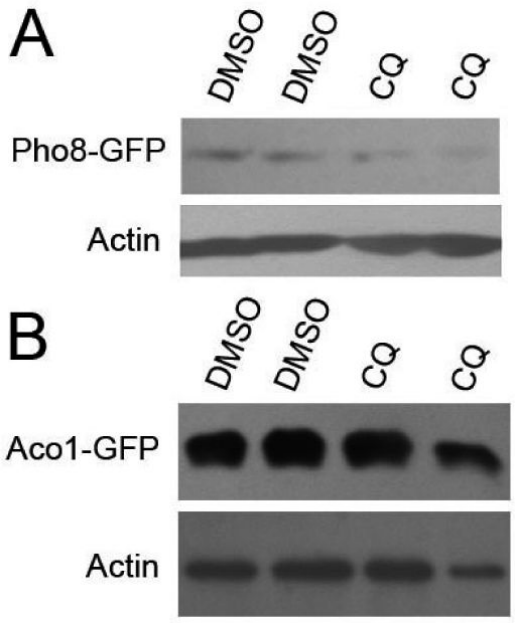

Figure S2. Western blotting analysis for the effect of CQ on expression of Pho8 and Aco1. A) protein level of Pho8-GFP is down-regulated by CQ. B) protein level of Aco1-GFP is not affected by CQ.

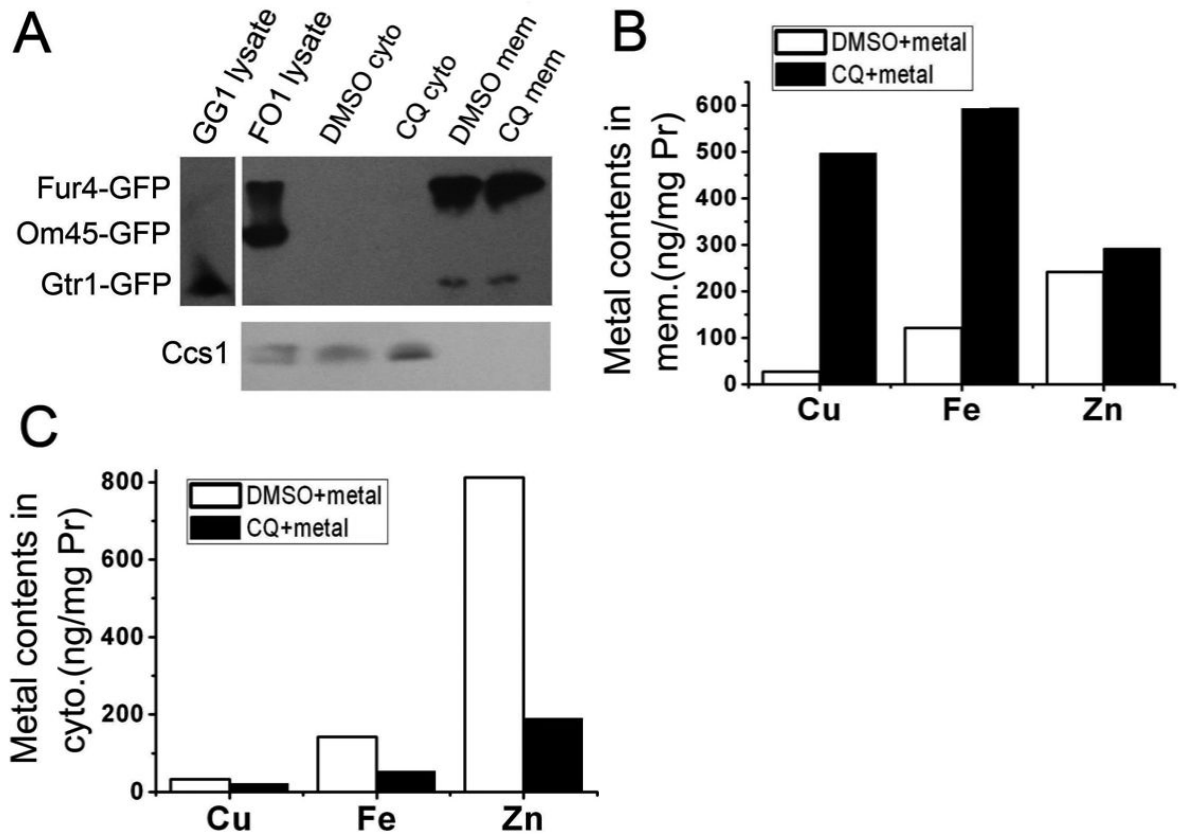

Figure S3. Western blotting and metal contents analysis of membrane/cytosol isolated from mixed trains of of the plasma membrane/cytosol isolated from mixed strains of FO1 and GG1. A) Western blotting analysis with GFP monoclonal antibody showed that the plasma membrane fractions from DMSO-treated mixed strains (DMSO mem) and CQ-treated mixed strains (CQ mem) contained the plasma membrane marker protein (Fur4-GFP) and a small quantity of vacuole membrane proteins (Gtr1-GFP as a marker); no mitochondrial membrane protein Om45-GFP was detected (the upper panel); probing with specific antibody to Ccs1p demonstrated that it could only be detected in the whole-cell lysate (FO1 lyaste) and cytosol fractions (DMSO cyto and CQ cyto; the lower panel). About 30 μg protein was loaded per lane. B) ICP-MS analysis indicated that CQ membrane possesses higher metal contents than the DMSO membrane; C) CQ cytosol possesses lower metal contents than its DMSO cytosol control. The results of metal contents in the fractions from these marked strains are highly similar to those obtained from the WT yeast experiment (Fig. 6 in the paper). No further duplicates were performed for the marked strains here.

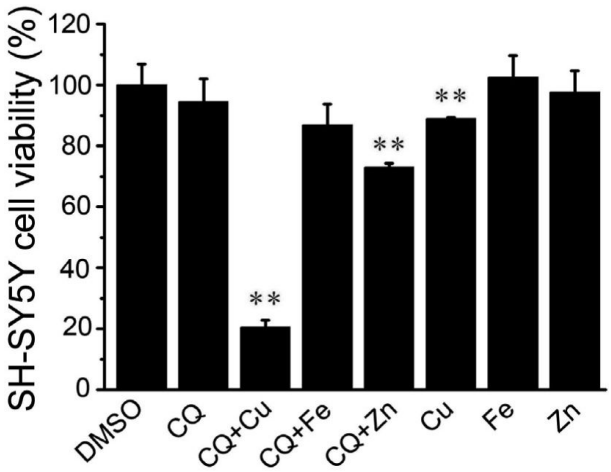

Figure S4. Effects of CQ and CQ-metal on SH-SY5Y cell viability. SH-SY5Y cells were treated with CQ (20 μM) and/or divalent metal ions (5 μM) for 24 h. Then cell viability was assayed with MTT method. CQ did not show obvious toxicity, but demonstrated synergetic toxicity with Cu or Zn. **$p < 0.01$, compared with DMSO control, unpaired t-test, $n = 3$.