SUPPLEMENTAL MATERIALS

Yeast Strains

Strains used to verify antibody specificity (Fig. S4) are derivatives of GA74-1A. Δ*cox4* (*MAT**a**, his3-11,15, leu2, ura3, ade8* Δ*cox4*::*TRP1*), Δ*rip1* (*MAT**a**, leu2, ura3, trp1, ade8,* Δ*rip1*::*HIS3MX6*), and Δ*qcr6* (*MAT**a**, his3-11,15, leu2, ura3, ade8* Δ*qcr6*::*TRP1*) were generated by replacing the entire open reading frame of the gene using PCR-mediated gene replacement (Wach *et al.*, 1994).

Table S1. Cld1p transmembrane domain predictions

TM prediction provider	Amino acids of predicted TM domains	Reference
DAS-TMfilter prediction server http://www.enzim.hu/DAS/DAS.html	None predicted	(Cserzo *et al.*, 2004)
TMpred http://www.ch.embnet.org/software/TMPRED_form.html	142-160, 225-241	(Stoffel *et al.*, 1993)
HMMTOP http://www.enzim.hu/hmmtop/	143-160	(Tusnady and Simon, 1998)
TMHMM Server v.2.0 http://www.cbs.dtu.dk/services/TMHMM/	None predicted	(Krogh *et al.*, 2001)
SPLIT http://split.pmfst.hr/split/4/	None predicted	(Juretic *et al.*, 2002)

Supplemental References

Cserzo, M., Eisenhaber, F., Eisenhaber, B., and Simon, I. (2004). TM or not TM: transmembrane protein prediction with low false positive rate using DAS-TMfilter. Bioinformatics *20*, 136-137.
Juretic, D., Zoranic, L., and Zucic, D. (2002). Basic charge clusters and predictions of membrane protein topology. J Chem Inf Comput Sci *42*, 620-632.
Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E.L. (2001). Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol *305*, 567-580.
Stoffel, W., Duker, M., and Hofmann, K. (1993). Molecular cloning and gene organization of the mouse mitochondrial 3,2-trans-enoyl-CoA isomerase. FEBS Lett *333*, 119-122.
Tusnady, G.E., and Simon, I. (1998). Principles governing amino acid composition of integral membrane proteins: application to topology prediction. J Mol Biol *283*, 489-506.
Wach, A., Brachat, A., Pohlmann, R., and Philippsen, P. (1994). New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast *10*, 1793-1808.

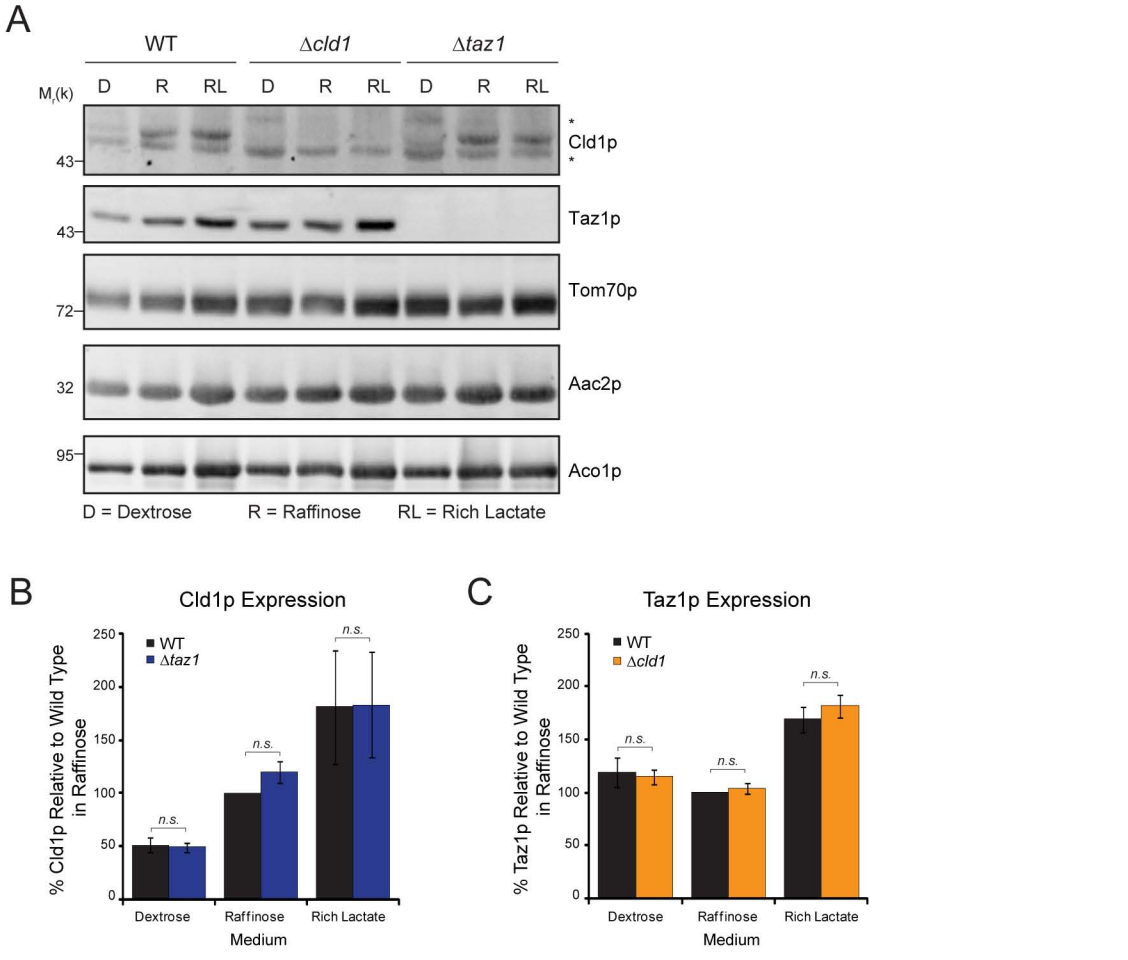

Figure S1. Cld1p and Taz1p expression is unaltered in CL remodeling pathway mutants. (A) Whole cell extracts from the indicated strains were grown in YP-dextrose(D), YP-raffinose (R), or rich lactate (RL), resolved by SDS-PAGE, and immunoblotted. (B) Cld1p or (C) Taz1p band intensities were quantified and plotted as the % protein relative to wild type grown in YP-raffinose (mean ± SEM, n=8). n.s. = differences not significant as determined by t-test

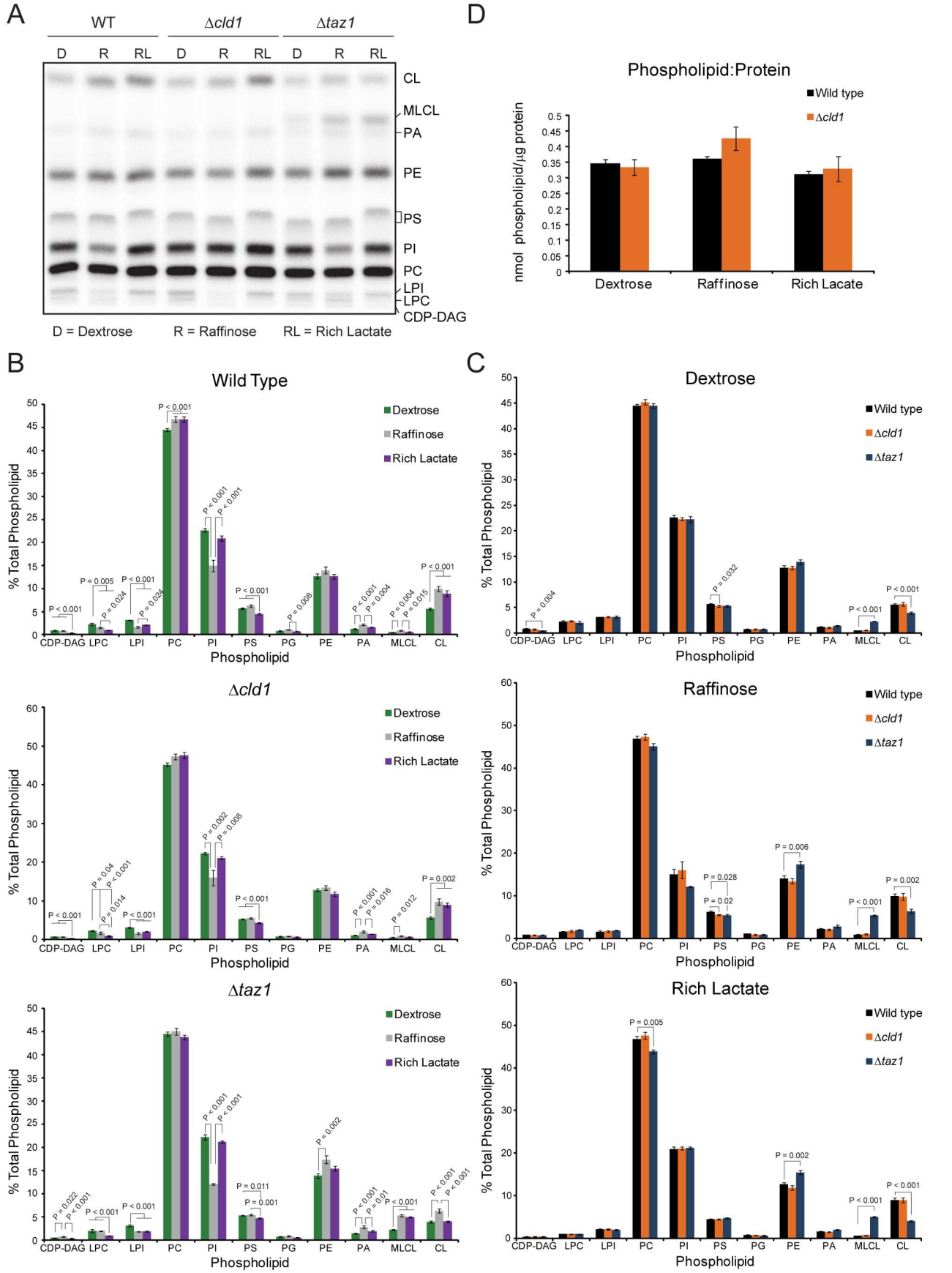

Figure S2. The effect of the available carbon source on mitochondrial phospholipids. (A) The full TLC plate shown in figure 6C, showing the separation of mitochondrial phospholipids from yeast grown in the indicated media after $^{32}P_i$ labeling. (B and C) Quantification of mitochondrial phospholipids (mean ± SEM, *n*=8). (B) Comparison of mitochondrial phospholipids from the indicated strain grown in the presence of different carbon sources. (C) Comparison of mitochondrial phospholipids from various yeast strains grown in the indicated carbon source. Significant differences determined by one-way ANOVA with pairwise comparisons. (D) The ratio of phospholipid:protein in wild type and Δ*cld1* mitochondria (mean ± SEM, *n*=3). Differences are not significant as determined by *t*-test.

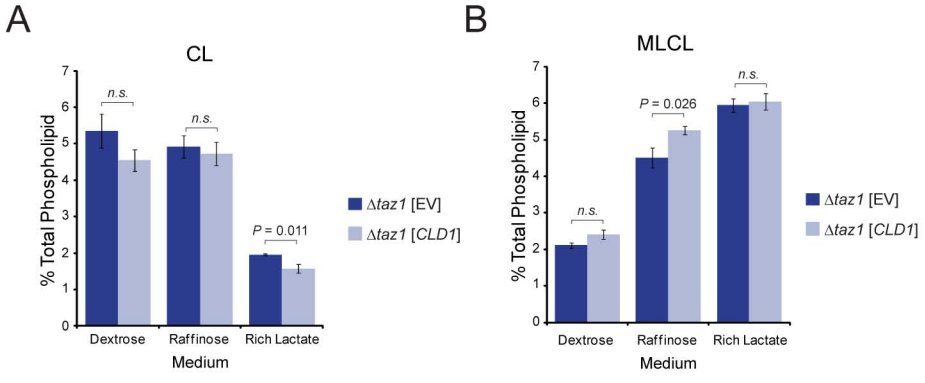

Figure S3. *CLD1* overexpression. (A) CL or (B) MLCL labeled with $^{32}P_i$ from Δ*taz1* yeast transformed with an empty vector (EV) or *CLD1* grown in the indicated media was separated by TLC and quantified (mean ± SEM, *n*=6). Statistical significance was determined by *t*-test. n.s. = differences not significant.

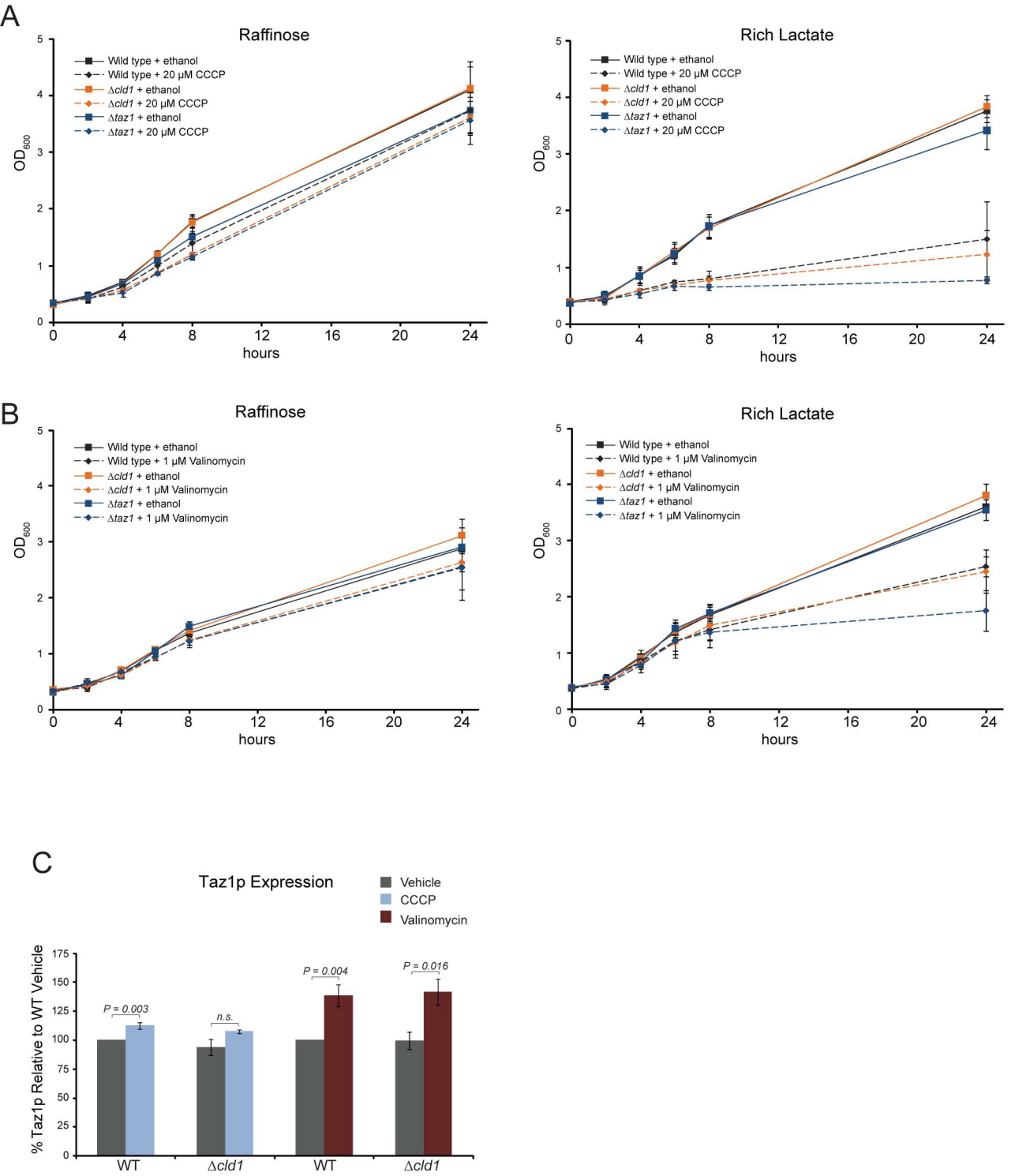

Figure S4. Ionophore treatment of yeast. (A and B) Cells were grown in YP-raffinose (left panels) or rich lactate (right panels) for 24 hrs at 30°C, diluted to 0.4 OD_{600}/ml, and treated with (A) 20 μM CCCP or an equal volume of ethanol or (B) 1 μM valinomycin or an equal volume of DMSO. The OD_{600} was measured at the indicated times. Data points represent the mean ± SEM, *n*=4. (C) Taz1p band intensities were quantified and plotted as the % protein relative to wild type grown in the presence of the vehicle (mean ± SEM, *n*=5). Statistical significance was determined by *t*-test. n.s. = differences not significant.

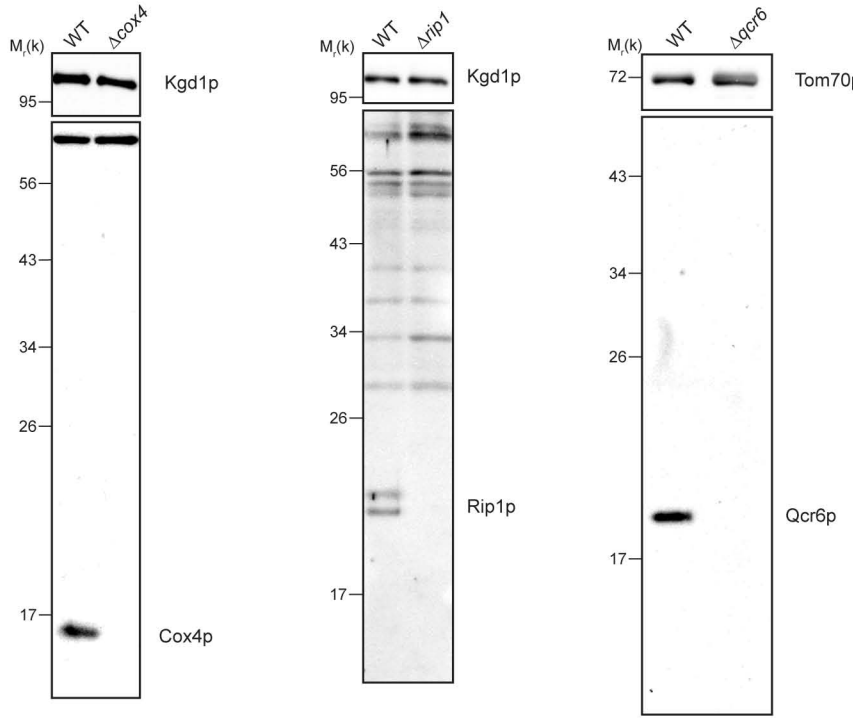

Figure S5. Antibody specificity. 25 µg of isolated mitochondria from the indicated strain were immunoblotted using antisera raised against Cox4p, Rip1p, or Qcr6p. Kgd1p or Tom70p served as a loading control