

Claypool et al., <http://www.jcb.org/cgi/content/full/jcb.201008177/DC1>

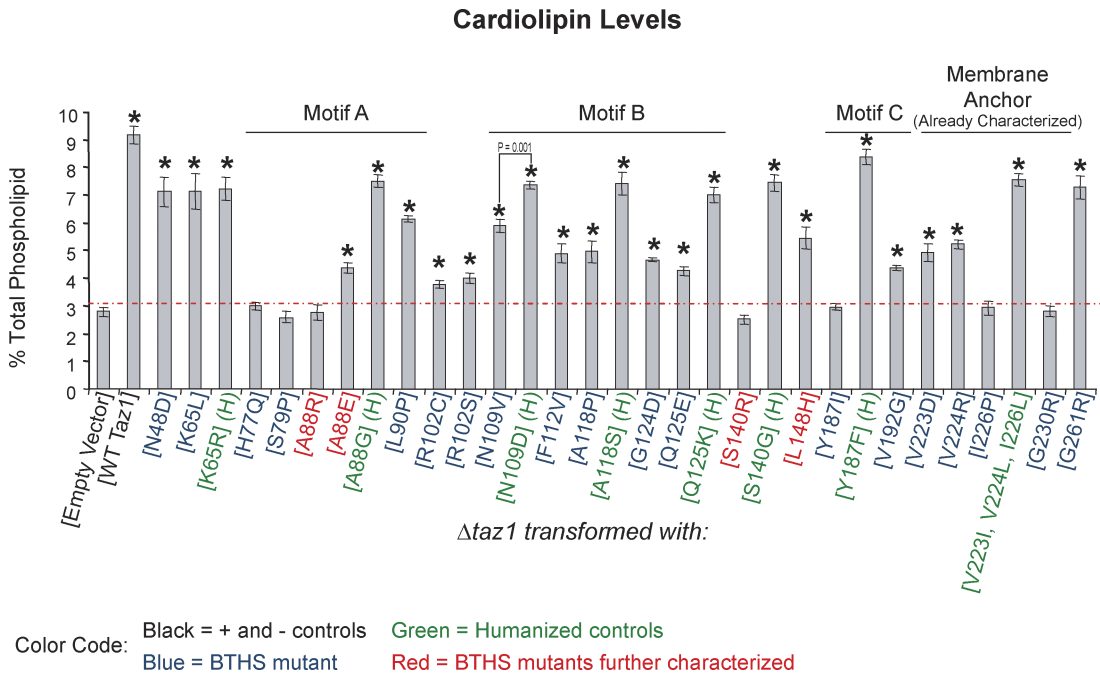


Figure S1. **18/21 BTHS mutations result in dysfunctional yeast *Taz1*p.** After steady-state labeling with ³²P, phospholipids were extracted from the indicated strains, separated by TLC, and identified by phosphoimaging. The relative abundance of CL was determined for each strain and is expressed as a percentage of the total phospholipid in each strain (means ± SEM; n = 3). The dashed red line indicates the highest level of CL detected in *Δtaz1* yeast transformed with the empty vector. Asterisks indicate a significant difference in the abundance of CL relative to *Δtaz1* yeast transformed with the empty vector (P < 0.001) as determined by one-way ANOVA with Holm–Sidak pairwise comparisons. BTHS mutations that occur within conserved acyltransferase motifs or an identified integral interfacial membrane anchor are indicated.

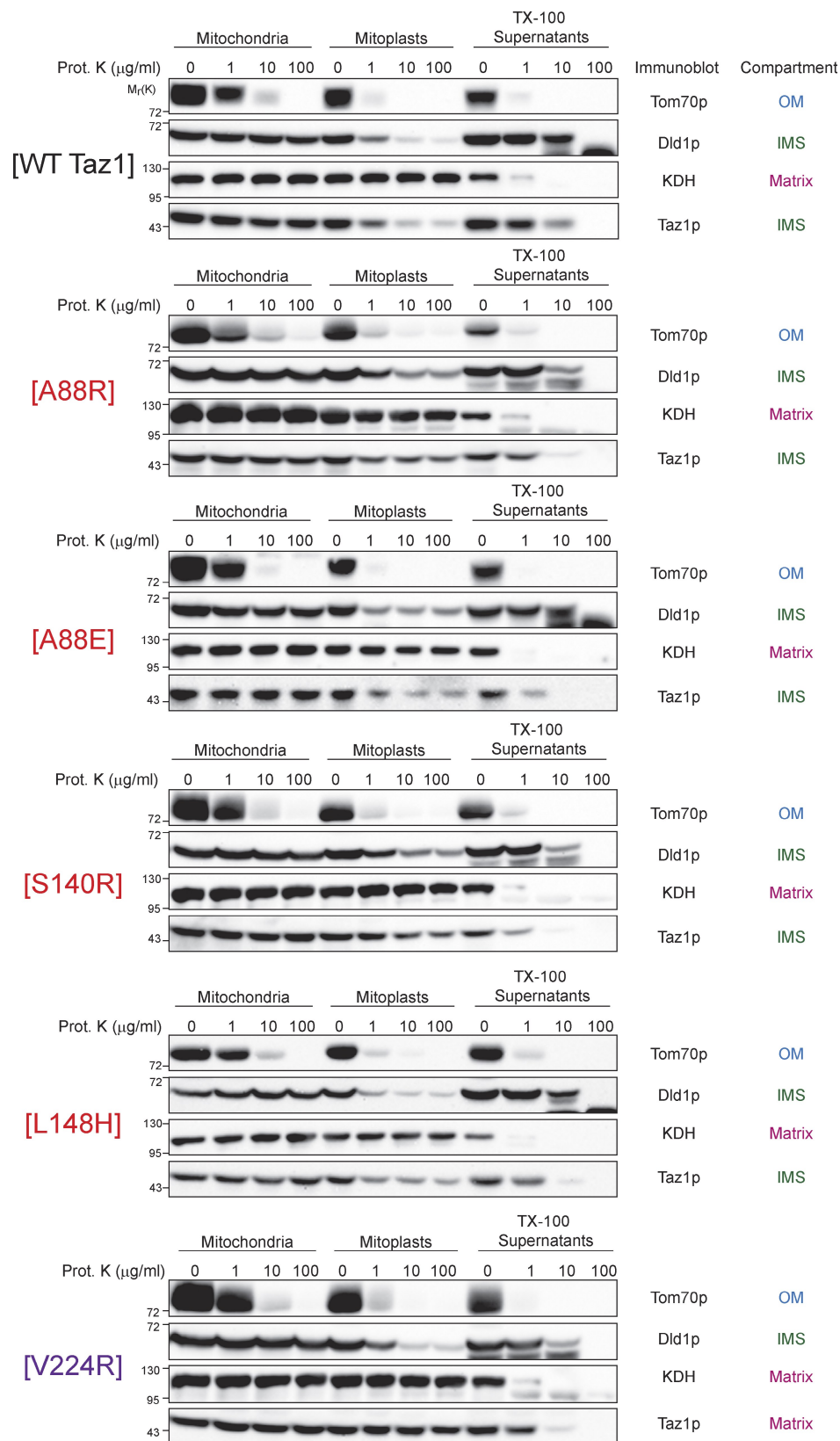


Figure S2. **The BTHS mutant tafazzins localize within mitochondria normally.** Submitochondrial localization of wt and BTHS mutant tafazzins. Intact mitochondria, mitochondria subjected to osmotic shock (mitoplasts), or mitochondria solubilized with 0.1% TX-100 were incubated alone or in the presence of the indicated concentration of proteinase K (Prot. K). 50 µg/lane wt Taz1p and 100 µg/lane BTHS mutants were resolved by SDS-PAGE and immunoblotted as indicated. The four BTHS mutants being characterized in the present study are shown in red. The previously characterized matrix-mislocalized BTHS mutant tafazzin is shown in purple. Relative molecular masses are shown on the left. KDH, α -ketoglutarate dehydrogenase. $n = 3$.

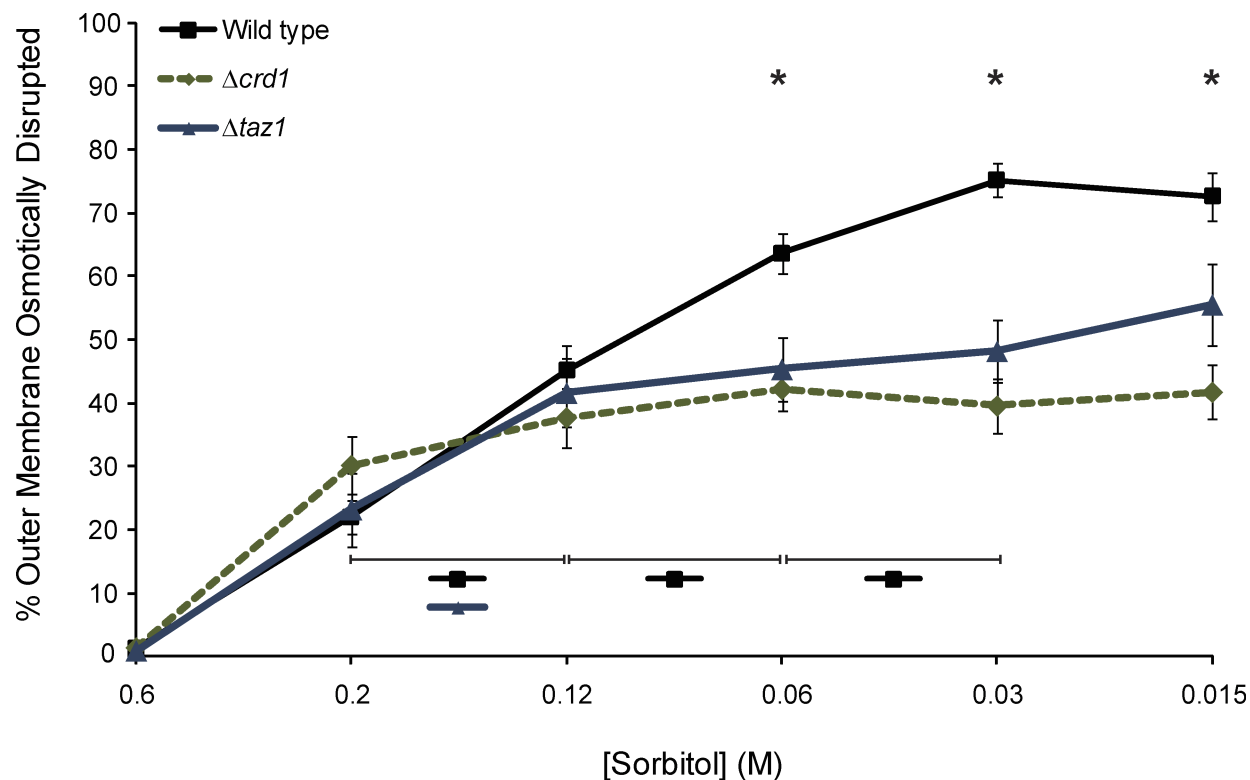


Figure S3. **CL-deficient mitochondria exhibit a defective swelling response.** Mitochondria were subjected to osmotic shock at the indicated final sorbitol concentration. Released cytochrome b2 and cytochrome c peroxidase, two soluble constituents of the IMS, was determined by immunoblotting after detection with a Versadoc and quantitation using the affiliated Quantity One software. The degree of osmotic shock is expressed as the percentage of OM osmotically disrupted and was calculated at each final sorbitol concentration as follows: $S/(S + P) \times 100$, in which S is the volume of tafazzin detected in the supernatant at a given final sorbitol concentration, and P is the volume associated with the pellet at the same final sorbitol concentration. Resultant data were collapsed for cytochrome b2 and cytochrome c peroxidase (means \pm SEM; $n = 5$). Statistical significance between treatments for each individual source of mitochondria are indicated by the presence of the corresponding symbol underneath the brackets between two treatments at $P \leq 0.05$ as determined by one-way ANOVA with Holm-Sidak pairwise comparisons. The asterisks indicate statistical significance of the wt mitochondria relative to both the $\Delta crd1$ and $\Delta taz1$ mitochondria at $P \leq 0.05$ as determined by one-way ANOVA with Holm-Sidak pairwise comparisons.

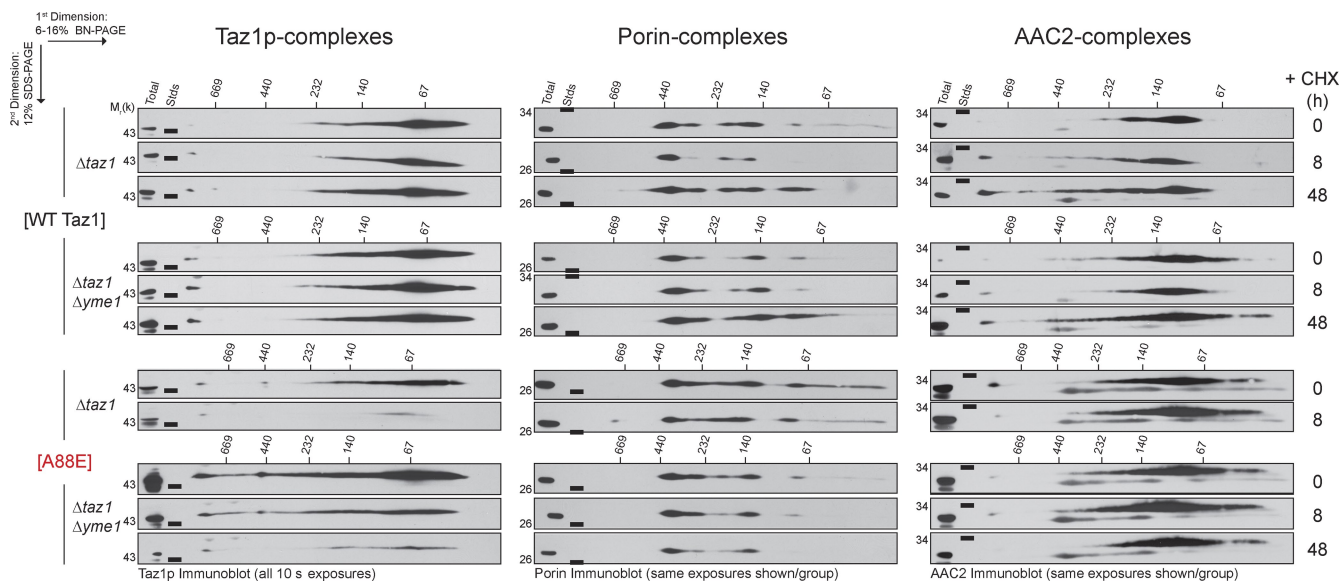


Figure S4. **BTHS mutant tafazzin complexes are unstable.** Mitochondria were harvested after incubation with cycloheximide (CHX) for the indicated times. 1.5% (wt/vol) digitonin extracts from mitochondria derived from the indicated strains were resolved by 2D BN/SDS-PAGE, and Taz1p, porin, and AAC2 complexes were detected by immunoblotting. 250 μ g ($\Delta taz1$ [A88E]) and 150 μ g (all the rest) were analyzed. The BTHS mutant characterized in the present study is shown in red. The migration of the high molecular weight markers for BN-PAGE is indicated at the top of each blot. Black boxes mark the position of the molecular weight standards (Stds) of the SDS-PAGE analysis.