

Supplementary Data 1. A core set of 740 high-confidence yeast mt proteins with subcellular localization data, along with 77 moderate-to-low confidence proteins, was defined based on multiple evidence sources.

Supplementary Data 2. Number of AP-MS purifications performed for each core mt bait protein.

Supplementary Data 3. Putative mt protein associations in the final YPEG-derived network.

Supplementary Data 4: Reciprocally detected mtPPIs from AP-MS and the 13,716-PPI network (Sheet 1), together with validated mtPPIs from the YPEG-derived network, are organized as follows: Sheet 2 lists interactions supported by both methods or by a single method exceeding the LLS threshold; Sheets 3 and 4 include interactions validated by external sources; Sheet 5 consolidates data from Sheets 2-4, CF-MS results from YPD, and reciprocal PPIs from 77 extended AP-MS baits; Sheet 6 provides examples of known interactions in major mitochondrial import and assembly pathways; and Sheet 7 lists the 1,648 OMM-non-mitochondrial interactions detected by both AP-MS and CF-MS.

Supplementary Data 5. PCCs of interacting mt proteins in the YPEG network, clustered by profile similarity into OMM (Sheet 1), IMS (Sheet 2), IMM (Sheet 3), and MM (Sheet 4).

Supplementary Data 6. Bulk RNA-seq showing mRNA changes in CoQ genes and respiratory complexes in orphan mutants vs. wild-type.

Supplementary Data 7. Interactions involving OMM proteins and non-mt compartments, with localization defined by consensus from multiple evidence sources.

Supplementary Data 8. Contact site resident proteins (linking mt and other organelles) from published studies and this study, along with their interactions with OMM proteins.

Supplementary Data 9. Putative multiprotein complexes identified from the YPEG network.

Supplementary Data 10. Protein complexes supported by AlphaFold 3D structure predictions.

Supplementary Data 11. Human orthologs of interacting yeast proteins from conserved complexes linked to human diseases.

Supplementary Data 12. Pearson correlation profiles of Rcf2 and Rcf3 with MICOS and respiratory chain components.

Supplementary Data 13. A draft conservation map of putative orthologous interactions in the YPEG mt network across all three domains of life (Sheets 1-2) was validated in select eukaryotic species using large-scale interactome studies (Sheet 3), the BioGRID database (Sheet 4), and AP-MS/CF-MS data from various human cell lines and mouse brain tissues (Sheet 5). Conserved orthologous mt interactors, including orphans, across 271 species are listed in Sheets 6-7.

Supplementary Data 14. Pearson correlation profiles of mouse orthologs of yeast mt interactors across brain regions (Sheet 1), and GO enrichment for orthologous yeast mt assemblies in the frontal cortex, striatum, and thalamus (Sheet 2).

Supplementary Data 15. List of strains, plasmids, primers, and antibodies used in the study.