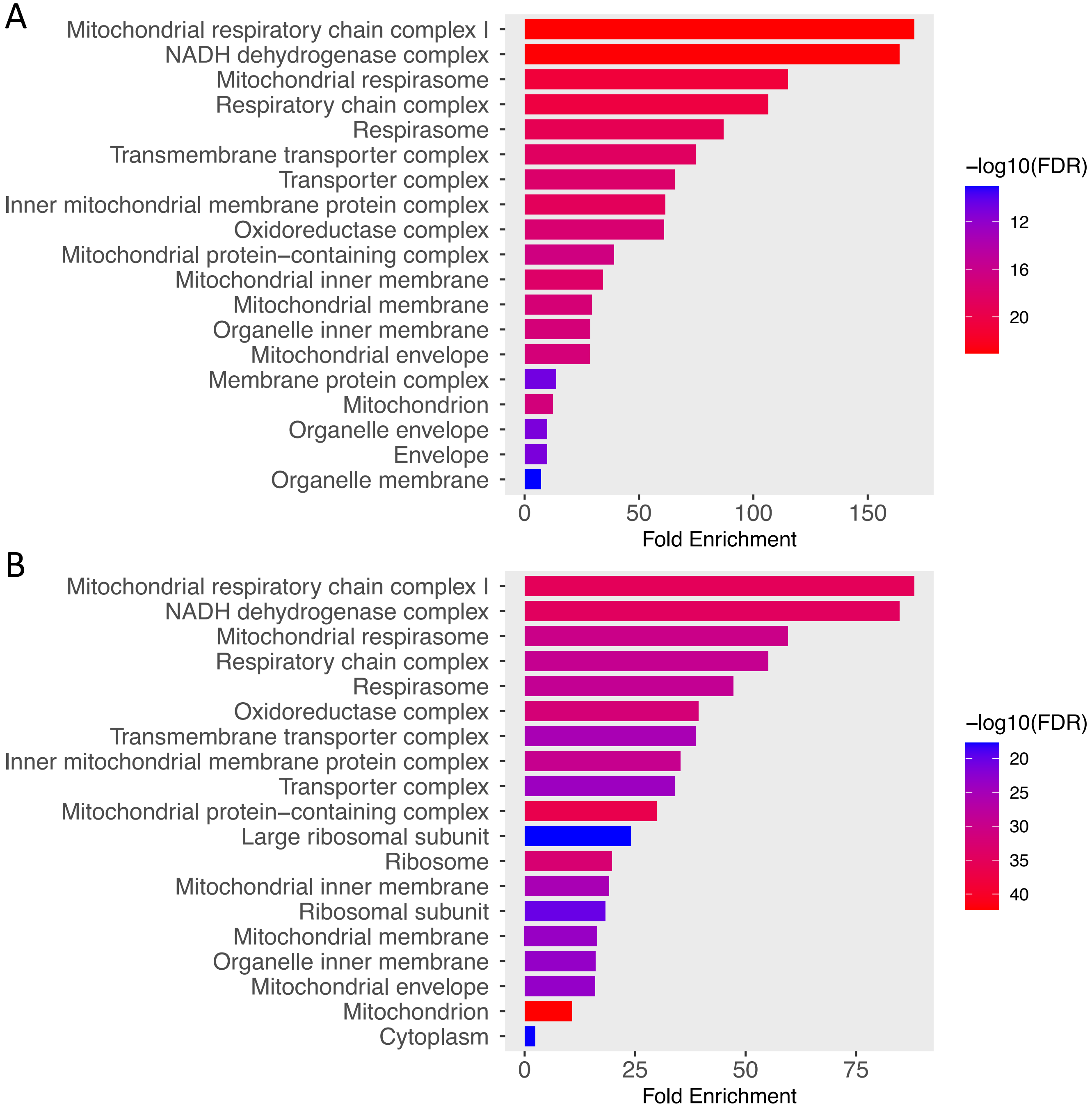

Figure S3. Functional analysis of proteins enriched by NDUFAF3 and TYKY co-immunoprecipitation

To perform the functional analysis of proteins enriched by NDUFAF3 (A) or TYKY (B) co-immunoprecipitation (coIP), we used ShinyGO v0.80 (https://bioinformatics.sdstate.edu/go80/) (1). Since ShinyGO relies on the Chlamydomonas reinhardtii genome annotation version 5.5, we first converted the protein IDs obtained from our IP experiments, which were based on version 6.1, to version 5.5. This conversion was carried out using the g:Profiler ID conversion tool (https://biit.cs.ut.ee/gprofiler/convert) (2). The conversion settings were as follows: Organism – Chlamydomonas reinhardtii; Target namespace – ENSP; Numeric IDs interpreted as ENTREZGENE.

For both NDUFAF3 and TYKY IP datasets, we selected proteins with a log2 fold change (log2FC) > 3. The resulting protein IDs were submitted to g:Profiler for conversion. In the case of the NDUFAF3 IP dataset, 50 out of 52 proteins were successfully converted to version 5.5 IDs. For the TYKY IP dataset, 164 out of 167 proteins were converted.

These converted IDs were subsequently analyzed in ShinyGO v0.80 using default parameters, with the "GO Cellular Component" ontology selected as the pathway database. Results were visualized based on Fold Enrichment and −log10(FDR) values.

Fold Enrichment quantifies the magnitude of enrichment, with higher values indicating a greater overrepresentation of a category and serving as an indicator of effect size.

P-values assess the statistical significance of the enrichment, with lower values indicating a lower probability that the observed result is due to random chance under the null hypothesis.

FDR q-values represent the False Discovery Rate-adjusted p-values, accounting for multiple hypothesis testing and reducing the likelihood of Type I errors.

References

1. Ge, S.X., Jung, D. and Yao, R., 2020. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics, 36(8), pp.2628-2629.
2. Liis Kolberg, Uku Raudvere, Ivan Kuzmin, Priit Adler, Jaak Vilo, Hedi Peterson: g:Profiler-interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update) Nucleic Acids Research, May 2023; doi:10.1093/nar/gkad347.