SUPPLEMENTARY FIGURES

"Duplexing Complexome Profiling with SILAC to Study Human Respiratory Chain Assembly Defects" by Páleníková et al.

This document contains three supplementary figures (Figure S1-S3) and two references.

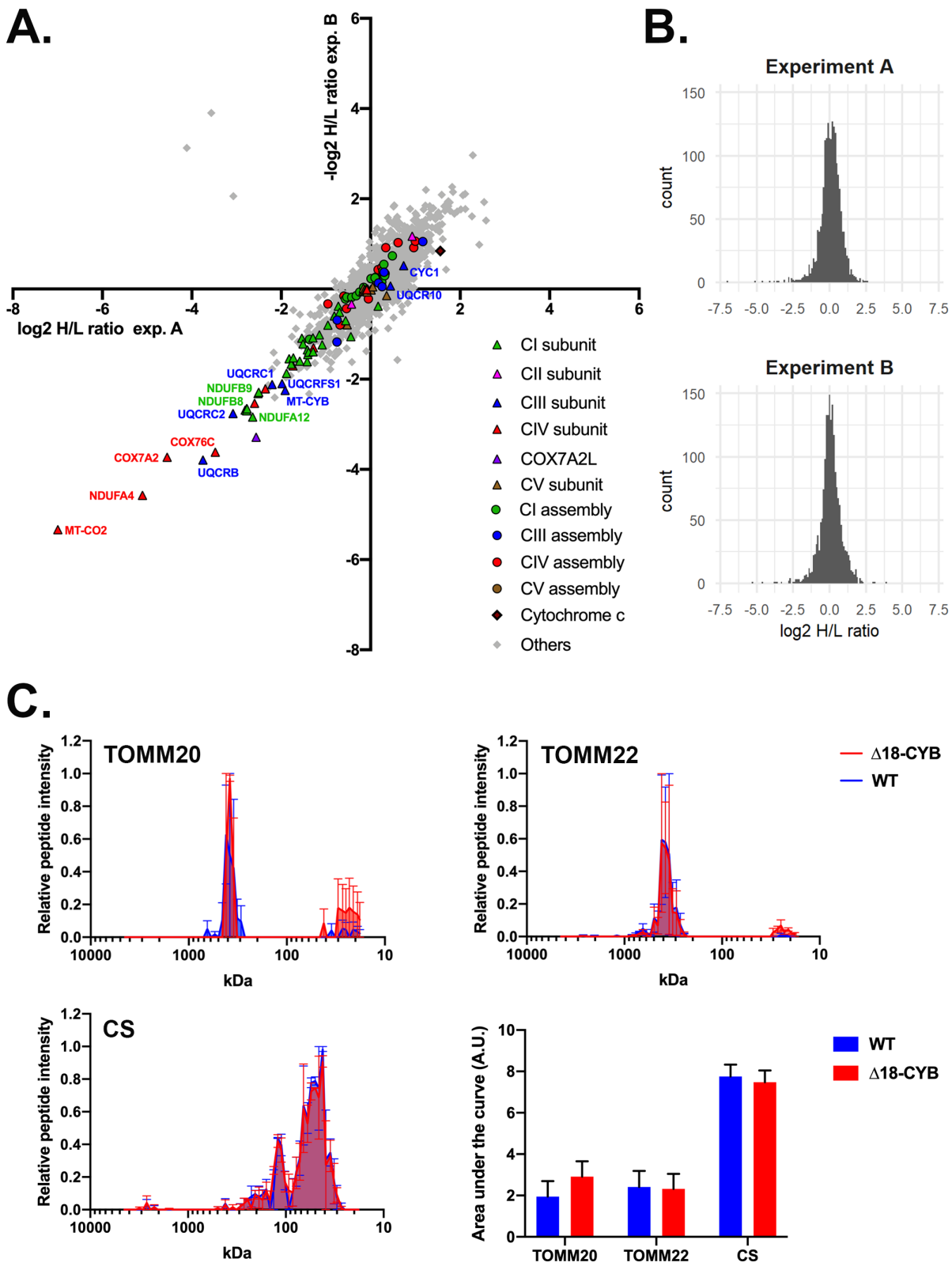

Figure S1: SILAC-based quantitative proteomics of WT vs. Δ18-CYB cybrids. (A) Scatterplot generated by plotting the $\log_2$ H/L ratios obtained by analysing the total mitochondrial fractions obtained from Experiment A (in the X-axis) and Experiment B (in the Y-axis). An aliquot of each one of the same mitochondrial extractions used for the SILAC-CP analysis was taken before BN-PAGE sample preparation. These were treated with 2% DDM and the total protein fractions were analysed

by MS, as described [1]. The fact that majority of the identified proteins gather around the axis origin indicates the correct mixing of the two differentially labelled cell types. The H/L ratios for the variable proteins are located along an imaginary 45º axis, indicating the reproducibility of the results in the two duplicate experiments [1]. (B) Histograms representing the frequency distribution of the logarithmic H/L ratios corresponding to the proteins detected in Experiment A (top graph) and Experiment B (bottom graph). These results indicate that most proteins showed a $\log_2$ H/L ratio of around zero, i.e., no variation in abundance between the H and L-labelled proteins. (C) SILAC-Complexome Profile graphs corresponding to proteins considered as mitochondrial mass markers, i.e., two members of the translocator of the mitochondrial outer membrane complex (TOMM20 and TOMM22) and citrate synthase (CS). The bar graph represents the quantification of the total peak area under the curves (AUC), in arbitrary units (A.U.), defined by the peptide intensity peaks for the indicated mitochondrial mass markers. The x-axis values were the slice number (1-64), and the y-axis values were the relative peptide intensity. The plotted values are the mean $\pm$ SEM (n = 2).

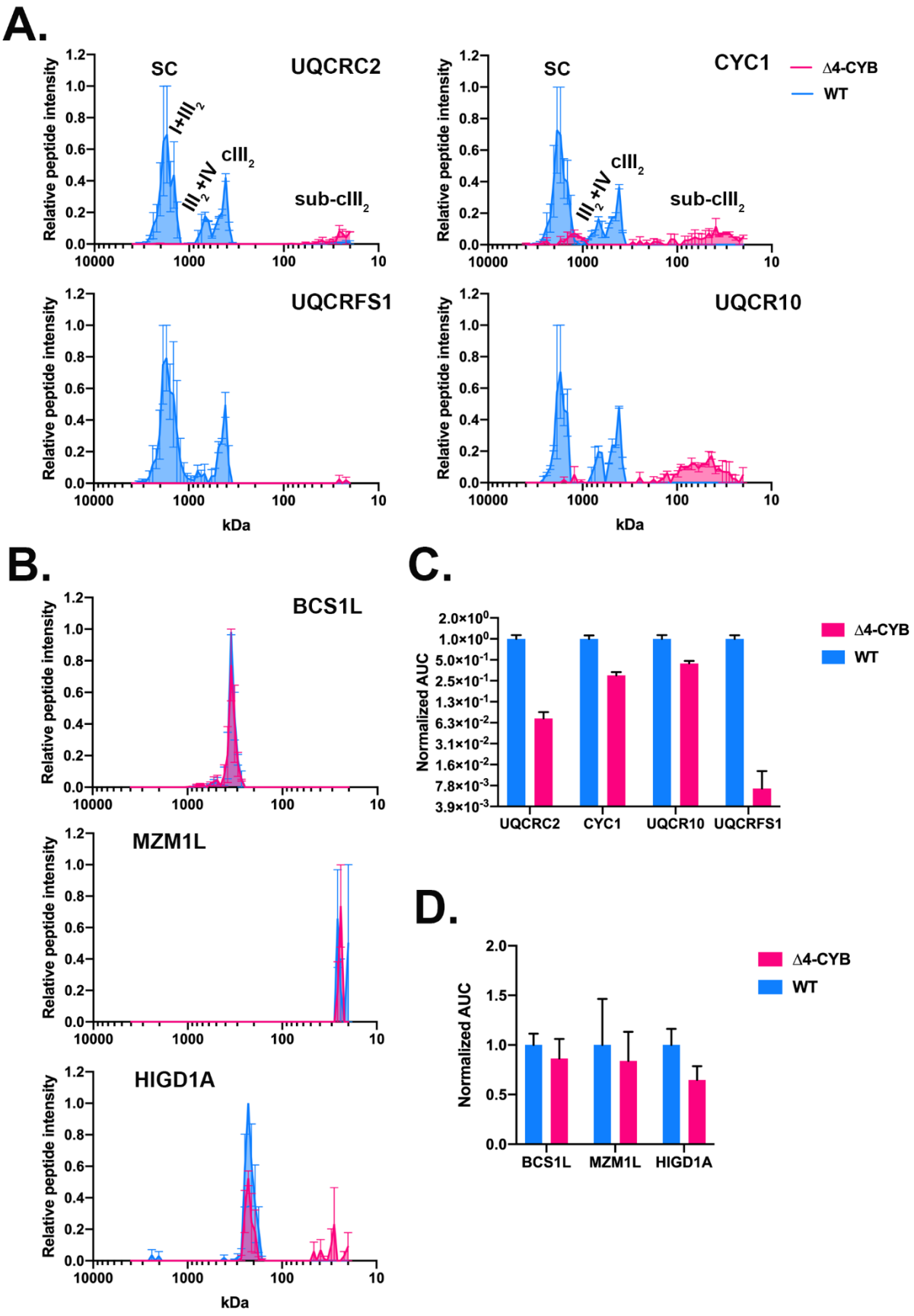

Figure S2: Relative distribution and abundance of $cIII_2$ structural subunit and assembly factors in Δ4-CYB cells in comparison with their isogenic WT control cybrids. Generated with the

data from [2] for comparison with Figure 3. (A) SILAC-CP analysis generated with the normalised (relative) common peptide intensity values of $cIII_2$ structural subunits UQCRC2, UQCRFS1, CYC1 and UQCR10. The values in the graphs are the mean ± SEM of the normalised peptide intensities derived from datasets #2 of the two samples from the duplicate reciprocal labelling experiments, separated in the same gel, in function of their calculated molecular size (kDa). (B) Complexome profiles generated in the same way as in (A) corresponding to the detected $cIII_2$ assembly factors BCS1L, MZM1L and HIGD1A. (C) Estimation of protein abundance by quantification of the total peak area under the curves (AUC) defined by the peptide intensity peaks for the indicated $cIII_2$ subunits. The x-axis values were the slice number (1-64), and the y-axis values were the relative peptide intensity. The AUC values for each protein were normalized to that of the WT. The graph shows the mean ± SEM (n = 2). (D) Protein abundance calculated as the normalised AUC for the indicated $cIII_2$ assembly factors, calculated as in panel C. The graph shows the mean ± SEM (n = 2). SC: supercomplexes $I+III_2+IV$ and $I+III_2$ (the peak at ~1,400 kDa corresponding to SC $I+III_2$ is specifically indicated); III_2+IV: supercomplex formed by the association of dimeric complex III ($cIII_2$) and complex IV; sub-$cIII_2$: subassembled species of lower molecular mass than mature $cIII_2$ (<485 kDa).

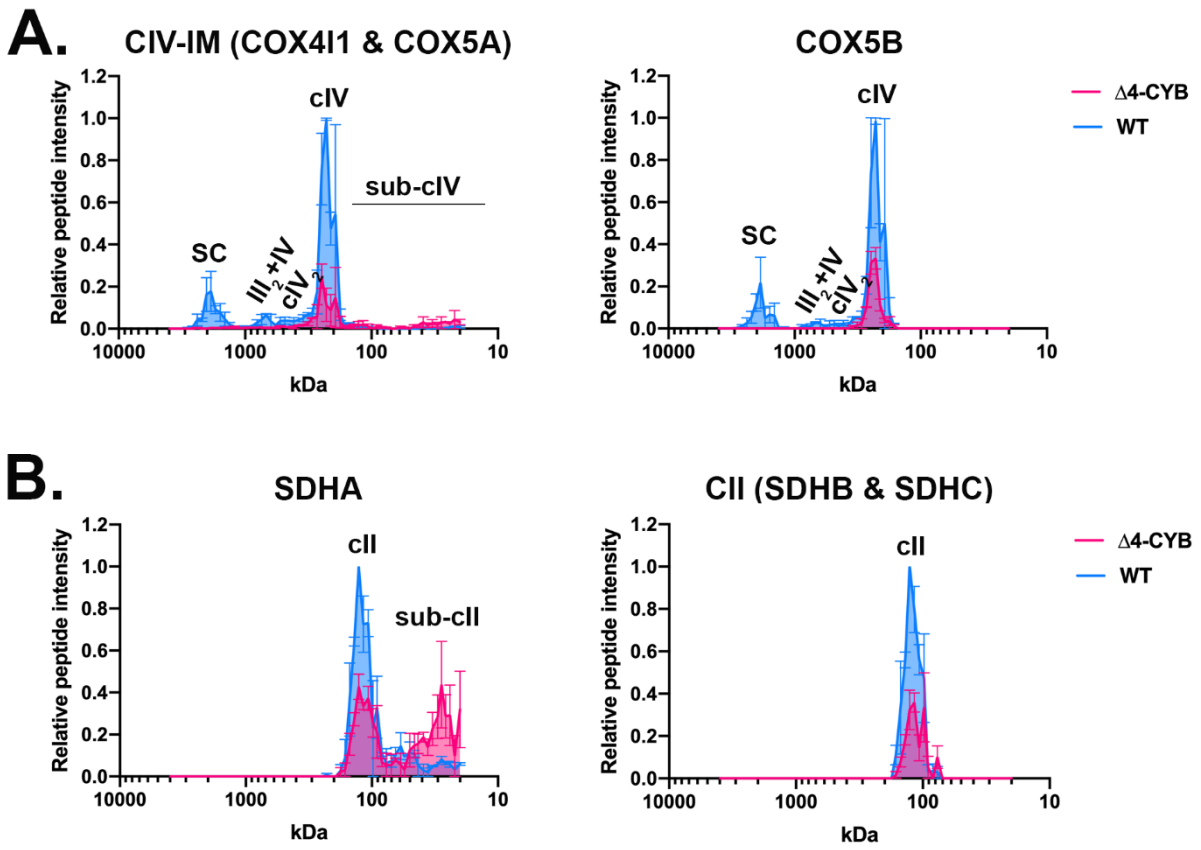

Figure S3: Relative distribution and abundance of cIV and cII structural subunits in Δ4-CYB cells in comparison with their isogenic WT control cybrids. Generated with the data from [2] for comparison with Figure 4. (A) SILAC-CP analysis generated with the normalised (relative) common peptide intensity values of (A) cIV and (B) cII structural subunits. The values in the graphs are the mean ± SEM of the normalised peptide intensities derived from datasets #2 of the two samples from the duplicate reciprocal labelling experiments, separated in the same gel, in function of their calculated molecular size (kDa). CIV-IM: complex IV initial module profile represented as the average of the normalised peptide intensity values corresponding to the COX4I1 and COX5A subunits. CII: complex II profile represented as the average of subunits SHDB and SDHC. SC: supercomplexes I+III_2+IV; III_2+IV: supercomplex formed by the association of dimeric complex III ($cIII_2$) and complex IV; cIV_2: dimer of cIV; sub-cIV: subassembled species of lower molecular mass than mature cIV (<214 kDa); sub-cII: subassembled species of lower molecular mass than mature cII (<123 kDa).

SUPPLEMENTARY FIGURE REFERENCES

[1] B. Andrews, J. Carroll, S. Ding, I.M. Fearnley, J.E. Walker, Assembly factors for the membrane arm of human complex I, Proceedings of the National Academy of Sciences of the United States of America 110(47) (2013) 18934-9.

[2] M. Protasoni, R. Perez-Perez, T. Lobo-Jarne, M.E. Harbour, S. Ding, A. Penas, F. Diaz, C.T. Moraes, I.M. Fearnley, M. Zeviani, C. Ugalde, E. Fernandez-Vizarra, Respiratory supercomplexes act as a platform for complex III-mediated maturation of human mitochondrial complexes I and IV, The EMBO journal 39(3) (2020) e102817.