

Fig. 1. BiFC assays with ProDH1, P5CDH, and OAT in *Nicotiana benthamiana*.

Representative confocal analyses of living epidermal cells of *N. benthamiana* showing transient expression of the infiltrated plasmid combinations indicated schematically on the left of each panel. The size of each cassette is proportional to the corresponding CDS length. YFP panels show YFP fluorescent signals; mCherry panels indicate the position of mitochondria; bright field panels show the cellular structure of the tobacco epidermal cells under white light; and merged panels show merged images from the three channels. Bars = 10 μ m.

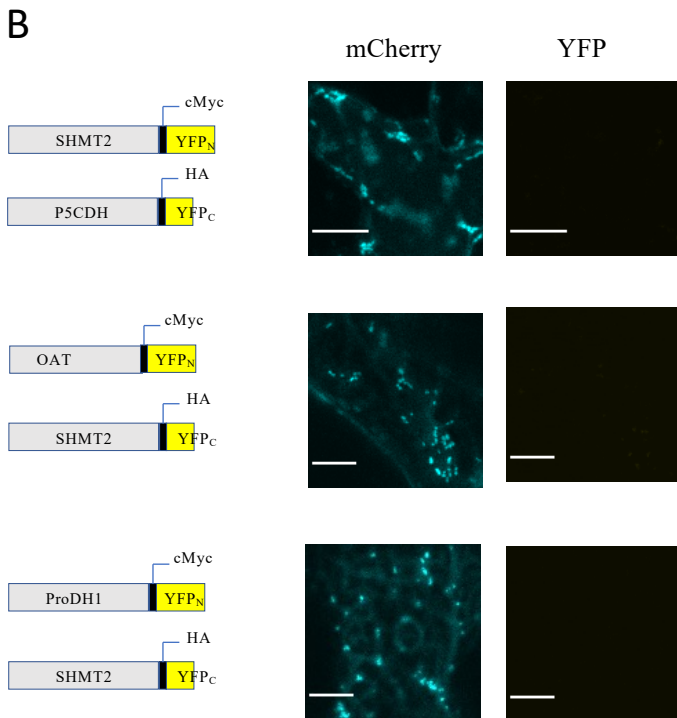
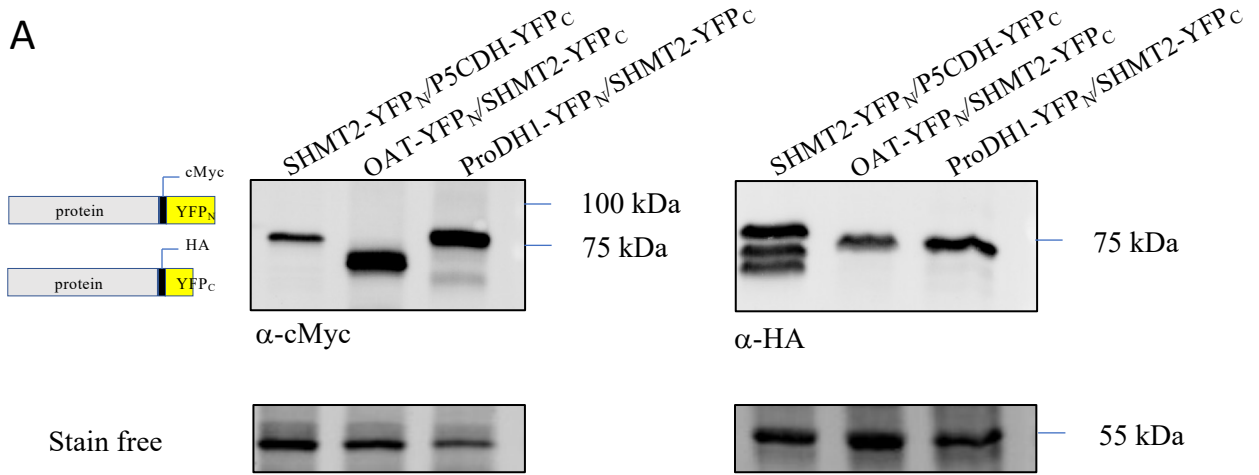


Fig. 2. BiFC assays of the interaction of either ProDH1, P5CDH, and OAT with the mitochondrial SHMT2 in *Nicotiana benthamiana*.

SHMT2 is a protein localized in mitochondria that was used as a negative control for BiFC. (A) Crude mitochondria were isolated from tobacco leaves infiltrated with different combinations of plasmids as indicated in the diagrams of the constructs and on the top of the membranes. Same amounts of mitochondrial proteins were loaded into SDS-PAGE gels (as shown by Stain Free Technology) and blotted onto PVDF membranes. Proteins were revealed using antibodies against MYC or HA tags. (B) The different combinations of ProDH1, P5CDH, and OAT with SHMT2 did not show any YFP fluorescence signal. mCherry signal revealed mitochondria. Bars = 10 μ m.

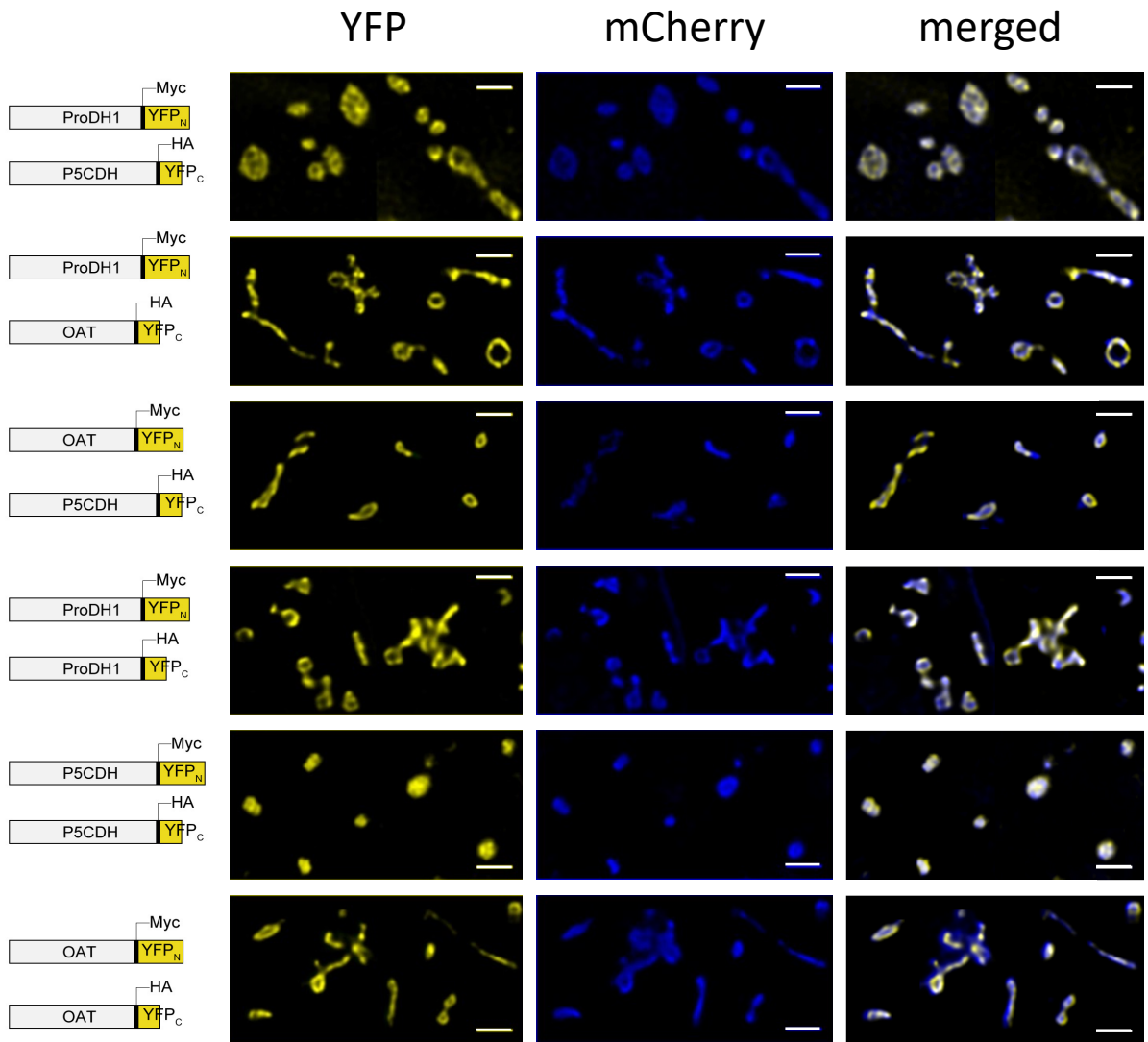


Fig. 3. BiFC assays with ProDH1, P5CDH, and OAT in fixed epidermal cells of *Nicotiana benthamiana*.

Z-spacing confocal analyses coupled with image deconvolution of fixed epidermal cells of *N. benthamiana* infiltrated with the plasmid combinations indicated on the left. YFP signals, which indicate the localization of protein interaction, are yellow. mCherry signals from the matrix of mitochondria are blue. The overlap between YFP and mCherry signals are shown in white. Bars = 1.5 μ m.

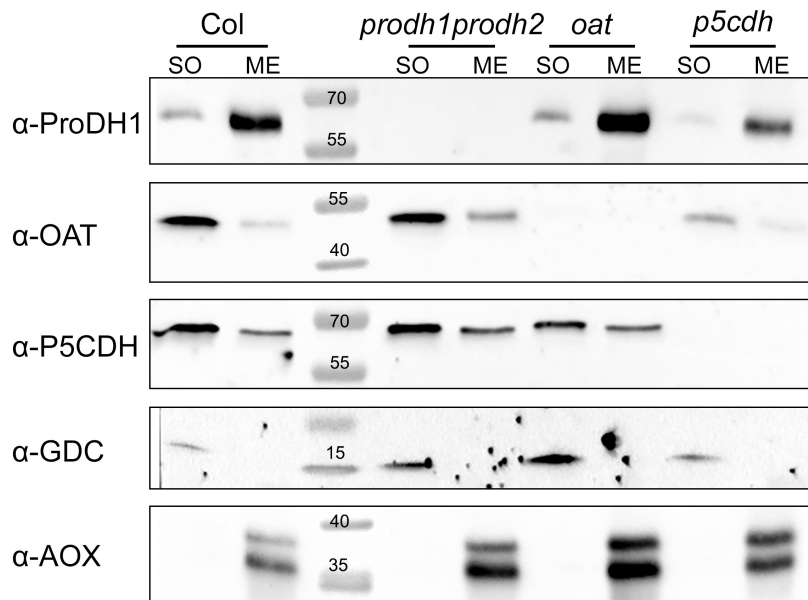


Fig. 4. Sub-mitochondrial localization of ProDH1, P5CDH, and OAT in *Arabidopsis thaliana* during DIS

Crude mitochondria were isolated from 4-week-old *A. thaliana* plants of the indicated genotypes after 5 days of DIS. Crude mitochondria were subjected to sonication and ultra-centrifugation to separate mitochondrial membranes from matrix. The same amounts of protein were loaded into each well of SDS-polyacrylamide gels which were blotted onto nitrocellulose membrane. Blots were probed with antibodies against alternative oxidase (α -AOX), glycine decarboxylase complex (α -GDC), α -ProDH1, α -P5CDH and α -OAT. ME, membrane fraction containing proteins from the mitochondrial inner and outer membrane; SO, soluble fraction containing proteins from the mitochondrial intermembrane space and matrix.

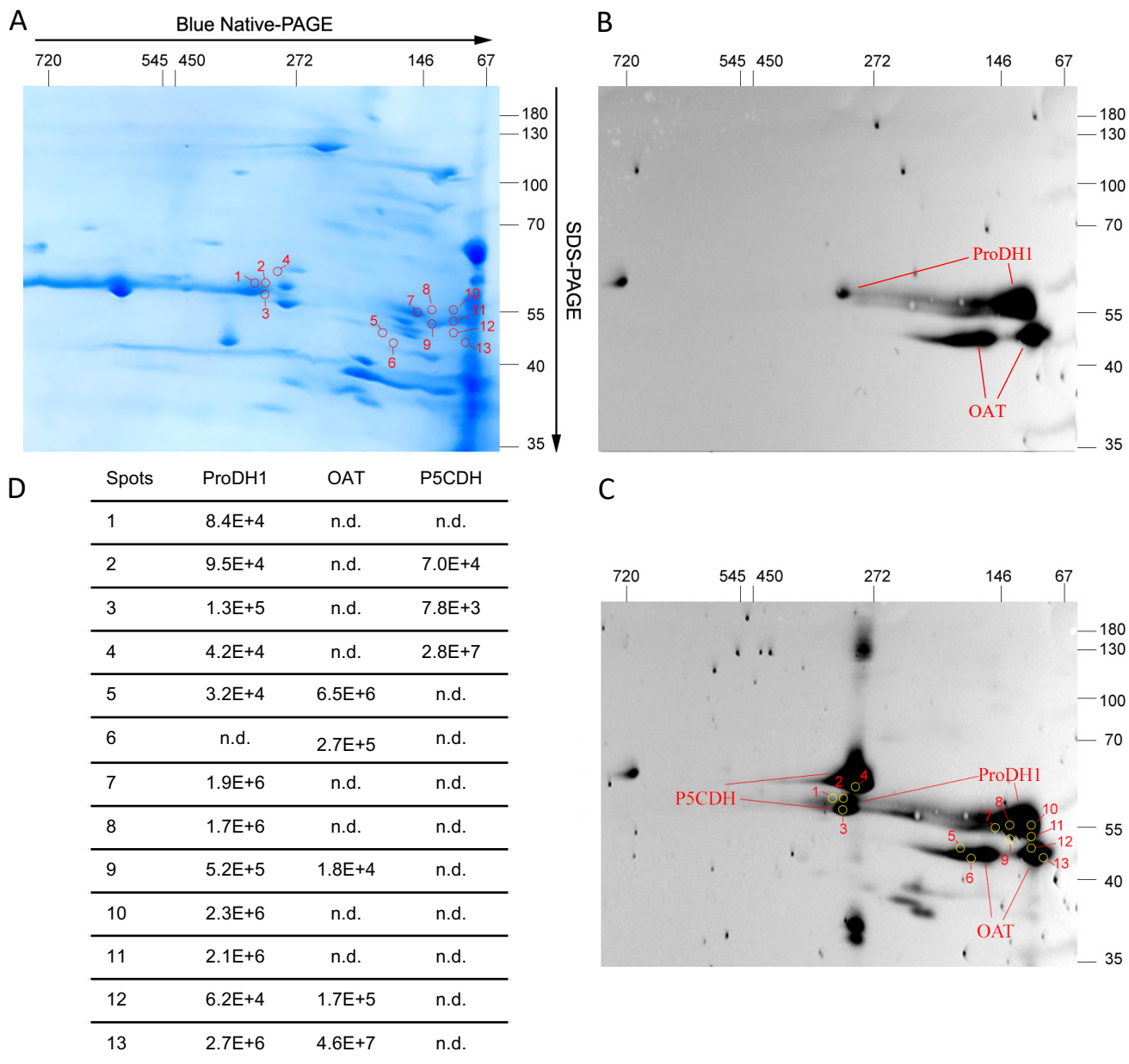


Fig. 5. Proteomic analysis of ProDH1, P5CDH, and OAT in mitochondrial fractions of *Arabidopsis thaliana* using 2D BN/SDS-PAGE, immunoblotting and MS

Crude mitochondria were extracted from Col-0 leaves of *A. thaliana* after 5 days of DIS and solubilized by 1% (w/v) digitonin. Proteins were separated by 2D BN/SDS PAGE, loading 1 mg of mitochondrial protein on each of two gels run in parallel. After electrophoresis, one gel was stained with Coomassie blue (A), and the other was used for western blotting (B-C). The masses of standard proteins are given on the top and the right of each gel image (in kDa). Primary antibodies against ProDH1 and OAT were first incubated with the membrane (B) and then P5CDH antibodies (C), as labelled. Gel spots excised from the Coomassie blue stained gel subjected to MS analysis are indicated by circles and numbered on the gel (A) and the membrane (C). iBAQ values obtained by MS analysis are shown in (D) for the abundance of ProDH1, OAT, and P5CDH peptides detected. N.d., not detected.

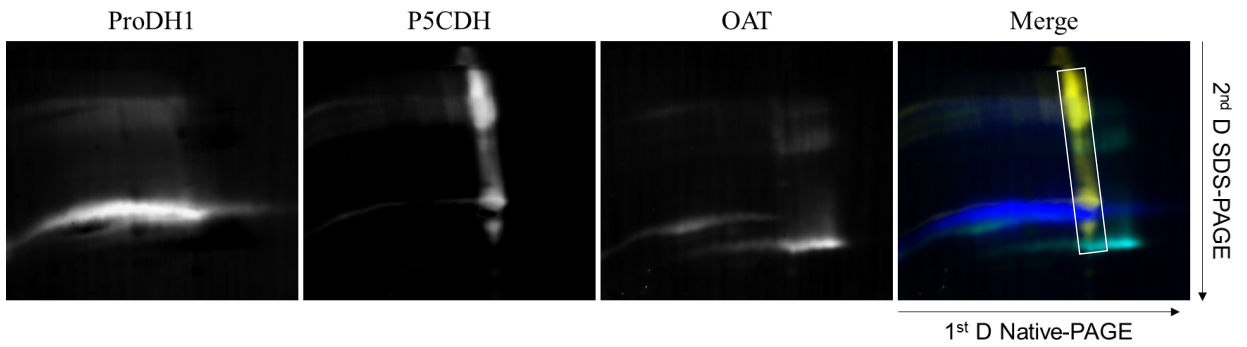


Fig. 6. ProDH1, P5CDH, and OAT expressed during DIS in *Arabidopsis thaliana* co-migrate on 2D clear native/SDS gels

Crude mitochondria were extracted from Col-0 leaves of *Arabidopsis* after 5 days of DIS, and solubilized by 1% (w/v) digitonin. Mitochondrial proteins (250 μ g) were separated by 2D clear native/SDS-PAGE, and the proteins transferred to a PVDF membrane, which was sequentially incubated with antibodies raised against ProDH1 (blue), OAT (cyan), and P5CDH (yellow) and analyzed using Image J. In the last right panel, the white rectangle indicates the co-migration of ProDH1, OAT and P5CDH to the same position in 1D electrophoresis.

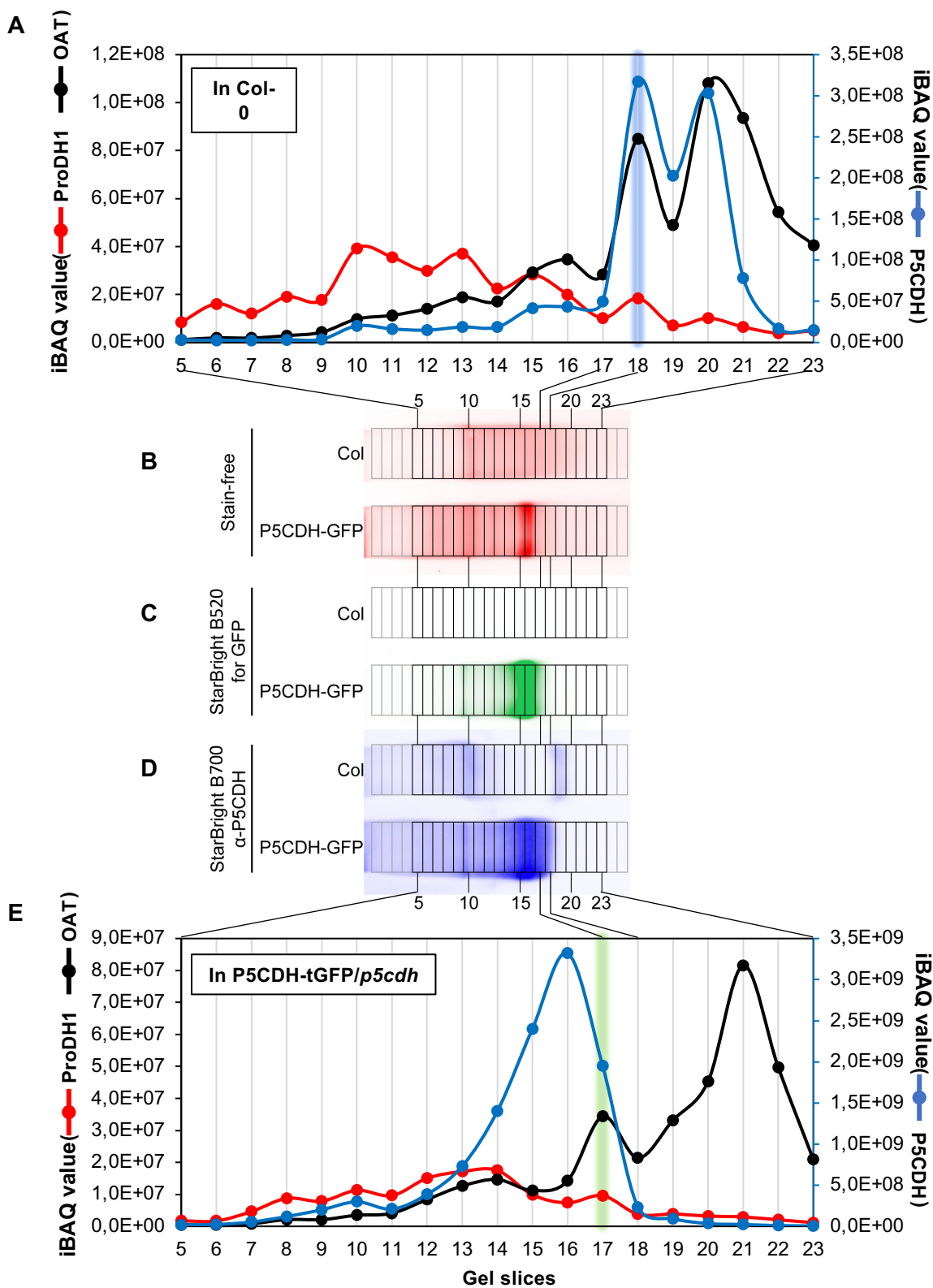


Fig. 7. Comparative complexome profiling of mitochondria from two Arabidopsis lines

Crude mitochondria extracted from Col-0 and P5CDH-tGFP/*p5cdh* Arabidopsis leaves after 5 days of DIS were solubilized with 1% (w/v) digitonin and separated by 1D clear native-PAGE (130 μ g of mitochondrial protein). After electrophoresis, gels were visualized using either the Stain-free channel for total protein (B) or the StarBright B520 channel for the GFP signal of the P5CDH-GFP protein (C). Gels were then blotted onto a membrane, which was probed with first an antibody against P5CDH and then with a StarBright Blue 700 fluorescent secondary antibody. Membranes were visualized using the StarBright B700 channel (D). Gel slices were cut as indicated in (B-D); gel slices 5 to 23 were used for label-free MS analysis. The iBAQ values of ProDH1, P5CDH, and OAT in Col-0 (A) and P5CDH-tGFP/*p5cdh* (E) are visualized in the alignments shown in parts B-D of the Figure.

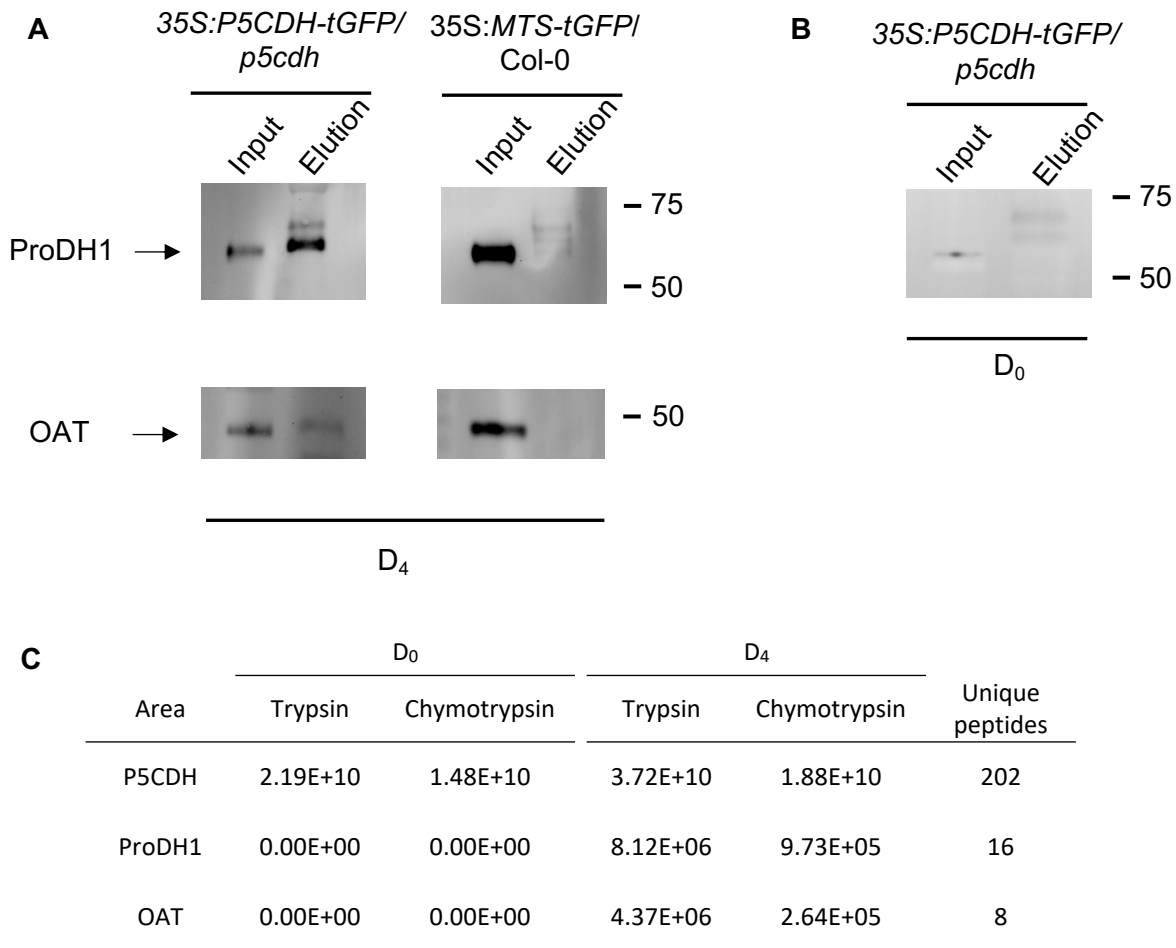


Fig. 8. Co-immunoprecipitation (Co-IP) of ProDH1 and OAT with P5CDH-tGFP

Co-IP using tGFP agarose beads from mitochondrial extracts at D4 (A) or D0 (B) of DIS from *35S:P5CDH-tGFP/p5cdh* and *35S:MTS-tGFP/col* leaves. Western blots of mitochondrial protein extracts (Input) and eluted fractions were probed with antibodies raised against ProDH1 and OAT. IBAQ values obtained by mass spectrometry analysis of Co-IP from mitochondrial extracts from either D0 or D4 *35S:P5CDH-tGFP/p5cdh* leaves are shown in (C).

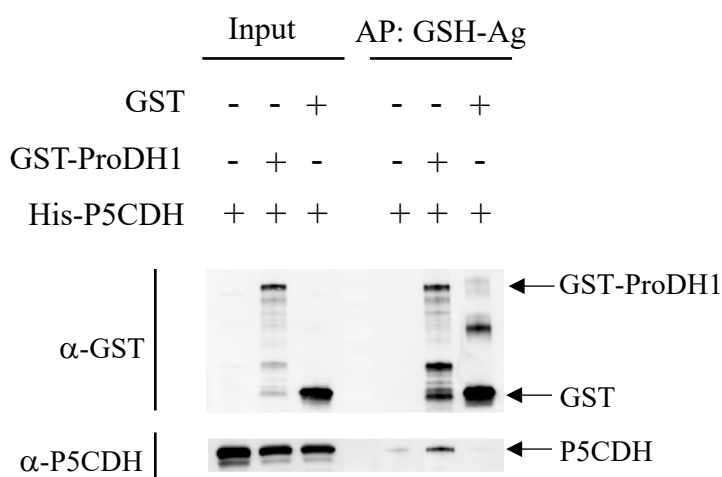


Figure 9: Co-purification of P5CDH with ProDH1 on GSH-agarose.

ProDH1-GST and His-P5CDH were co-overexpressed in BL21(C41) *E. coli* cells. Total protein extracts (Input) were loaded onto GSH-agarose. After washing, proteins were eluted with Laemmli buffer. Inputs and eluted proteins from GSH-agarose (AP:GSH-Ag) were revealed by western blot analysis using antibodies raised against P5CDH or GST. Total protein extracts from bacteria over-expressing free GST and His-P5CDH were used as negative control.