

Supplementary Fig. S1. Proline sensitivity of p5cdh was complemented in 35S:P5CDH-tGFP/p5cdh plants.
Seeds of different genotypes (as indicated in the Figure) were sterilized, stratified at $4^{\circ} \mathrm{C}$ for 72 h and grown onto a MS/2 medium supplemented without (A) or with 25 mM proline ( $B$ ) for 7 days.
mCherry


Bright Field



Empty- $\mathrm{YFP}_{\mathrm{N}}$
ProDH1-YFP ${ }_{c}$

Empty-YFP ${ }_{N}$
P5CDH-YFP

Empty-YFP ${ }_{N}$ OAT-YFP ${ }_{C}$

P5CDH-YFP ${ }_{N}$ OAT-YFP ${ }_{C}$

P5CDH-YFP ${ }_{N}$ ProDH1-YFP ${ }_{C}$
ProDH1-YFP
Empty-YFP

P5CDH-YFP
Empty-YFP ${ }_{C}$

OAT-YFP $_{N}$
Empty-YFP



Supplementary Fig. S2. Controls and additional BiFC assays in Nicotiana benthamiana.
YFP channel shows YFP fluorescent signals; mCherry channel indicates the location of mitochondria; bright field channel shows the cellular structure of tobacco epidermal cell under white light; merged channel shows picture merged with other three channels. Bar $=10 \mu \mathrm{~m}$.


Supplementary Fig. S3. Protein expression of BiFC-infiltrated Nicotiana benthamiana leaves. (A) Representative diagrams of constructs used for BiFC assays. Cassettes are presented in 5' to $3^{\prime}$ direction. The size of each cassette is based on their nucleic acid sequence length. Gray box, insertion of the CDS fragment of each construction; Myc, c-myc epitope tag; HA, hemagglutinin epitope tag; $\mathrm{YFP}_{\mathrm{N}}, \mathrm{N}$-terminal fragment of eYFP; YFP ${ }_{C}$, C -terminal fragment of eYFP.
(B-F) Total proteins were extracted from tobacco leaves infiltrated with different combinations of plasmids indicated on the top of each western-blot. Same amount of proteins was loaded into SDS-PAGE gels. Proteins were then transferred onto a nitrocellulose membrane and revealed using antibodies raised against c-Myc or HA tags. Constructs with positive YFP signal are labelled with yellow backgrounds. The theoretical molecular weight of B4-ProDH1 is 73.7 kDa; B5-ProDH1, 65.6 kDa; B4-OAT, 70.9 kDa; B5-OAT, 62.8 kDa; B4-P5CDH, 80.5 kDa; B5P5CDH, 72.4 kDa .


AT1G52000.1_Mannose-binding lectin superfamily protein






Supplementary Fig. S4. Migration pattern of selected mitochondrial proteins which are not affected by the presence of P5CDH-tGFP.
The iBAQ values of each protein in Col-0 and P5CDH-tGFP/p5cdh in different gel slices are shown.



Gel slices

## Supplementary Fig. S5. Complexome profiling for SDH1-1.

The iBAQ value of ProDH1, P5CDH, OAT, and Succinate dehydrogenase 1-1 (SDH1-1) in Col-0 (A) and in P5CDH-tGFP/p5cdh (B) are shown. The iBAQ values of P5CDH is shown on the right $y$-axis, iBAQ values of the other proteins are shown on the left $y$-axis.

