

Supplemental Data. Peng et al. (2009). Efficient operation of NAD(P)H dehydrogenase requires supercomplex formation with photosystem I via minor LHCI in *Arabidopsis*.

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AtNDH18  1  --MATVTILSPKSIIPKVTDKFGARVSDQIVN---VVKCGKSGRRRLKLAKLVS-----
GmNDH18  1  --MALCSSLVSLVSPIKLTSKSFSLPTVPSWHG---ILIKNNTSTRFLLK-----
NtNDH18  1  --MADSVTISLLSTVPVNPVLSLKSGESQLKYNFLFRGNQCSISLKIIGTKCSGMRSS--
OsNDH18  1  MMSSSSSLVSAISASPSATAKARLRHDDAATATGAAAAAAVAARQLKTEHGGCCCCRSRG
ZmNDH18  1  ---MSSLSSGISASPSAAPSTARRRHDAAPQQLRVKAAARPQLLHAGRRRSVGRPPGG

          ↓
AtNDH18  49  -----AAGLSQIEPDINEDPIGQFETNSIEMEDFKYGYDGAHTYVEGEVQKGTFWG
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NtNDH18  57  ----TRLYAGLTEIEPDINEDPVDRWRNNGIEIEDFVFGKYDDHHTYFESE-DKATFWE
OsNDH18  61  RAVVARAGPGPLTEIEPDLNEDPIDKWRNNGVSPDDFEYGVYDGHHTYNETQ-DKKGFWE
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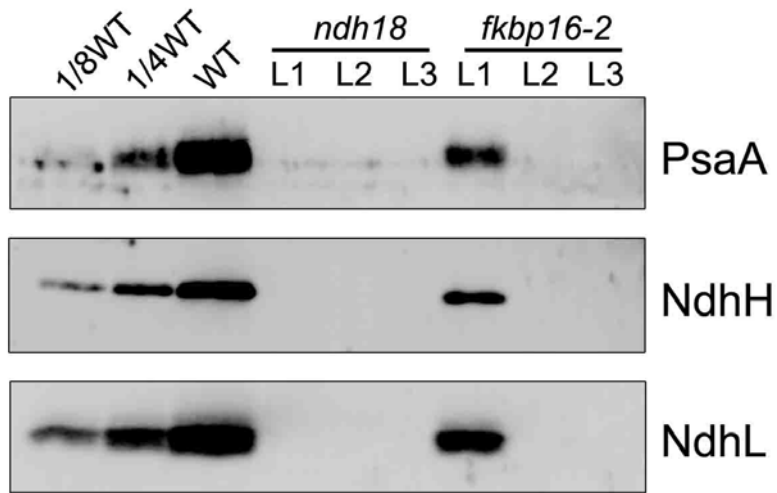
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NtNDH18  111  SIKEDYEAVAPPRGFQGLISWLFPPAIALGMYFNVPGEYLYIGAAVFTIVFCIIEMDKPS
OsNDH18  120  DVSEWYQEAEPQGFQALISWLFPPAIALGMAFNVPGEYLYIGAAVFTIVFCIEMDKPD
ZmNDH18  116  DVSEWYQEAEPQGFQALISWAFPPAIALGMAFNVPGEYLYIGAAVFTIVFCIEMGKPD

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GmNDH18  155  KPHHFEPHIYNMERGARDKLIINDYNTMSIWFNFKYGDLDWDTTIPIKDDIMKR-
NtNDH18  171  EAHNFEPQIYNMERGARDKLIINDYNTMDIWFNFKYGDVWDFTVKQLQKDDIMKR-
OsNDH18  180  KPHNFEPQIYMMERSARDKLIADYNTMDIWFNFKYGEVLDWFTVD-RRDEIVKSS
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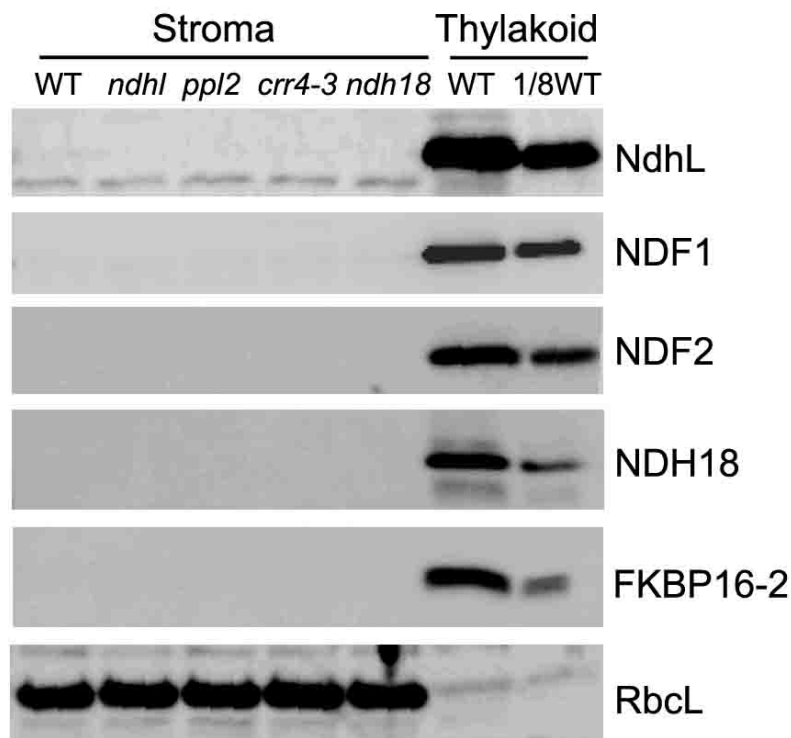
Supplemental Figure 1. Amino Acid Sequence Alignment of NDH18.

Protein sequences were aligned using the ClustalW program with default settings (<http://clustalw.ddbj.nig.ac.jp/top-e.html>). Black boxes indicate strictly conserved amino acids, and gray boxes indicate closely related amino acids. The bar above the sequences indicates the transmembrane domain in At NDH18. The arrow indicates the predicted cleavage site for the plastid-targeting signal. The sequences are named for each organism as follows: At NDH18, *Arabidopsis thaliana* (At5g43750); Gm NDH18, *Glycine max* (accession number CD394214); Nt NDH18, *Nicotiana tabacum* (EB679832); Os NDH18, *Oryza sativa* (Os01g0929100); Zm NDH18, *Zea mays* (DV514173).



Supplemental Figure 2. NDH-PSI Supercomplex Content in the *ndh18* and *fkbp16-2* RNAi lines.

Thylakoids isolated from the wild type (WT), *ndh18* and *fkbp16-2* RNAi lines were subjected to BN-PAGE analysis. After electrophoresis, the high-molecular-weight region corresponding to Bands I and II was excised from the BN gel, and the proteins were denatured in the gel with SDS sample buffer. The proteins were further separated by 12.5% SDS-PAGE and immunodetected with specific antibodies against PsaA, NdhH and NdhL.



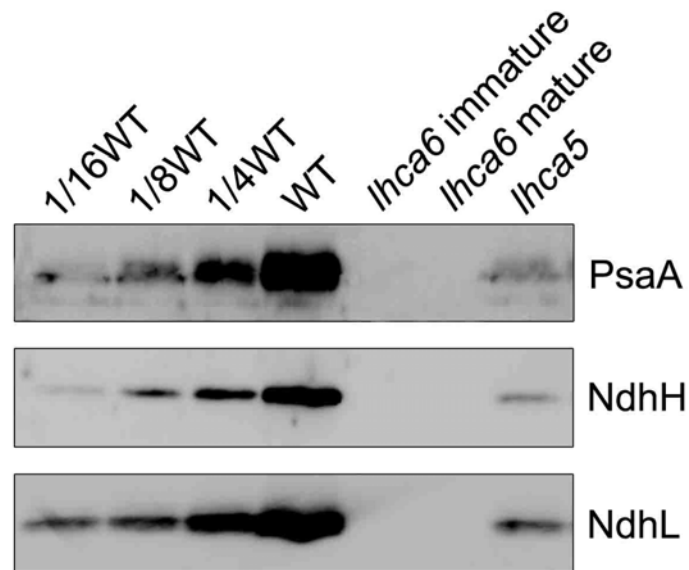
Supplemental Figure 3. Localization Analysis of NDH Subunits in Chloroplasts of the Wild Type (WT), *ndhl*, *ppl2*, *crr4-3* and *ndh18* Mutants.

Isolated chloroplasts were osmotically ruptured in a buffer containing 20 mM HEPES/KOH (pH 7.6), 5 mM MgCl₂, and 2.5 mM EDTA. Thylakoid membranes were pelleted by centrifugation (17,600 × g for 20 min) and resuspended in the same buffer. The concentration of the stroma protein was determined with a Bio-Rad Protein Assay Kit (cat. No. 500-0006). Immunoblotting was performed with antibodies against NdhL, NDF1, NDF2, NDH18, FKBP16-2, and RbcL proteins. Thylakoid proteins were loaded on an equal chlorophyll basis and 2μg stroma protein was loaded on each well.



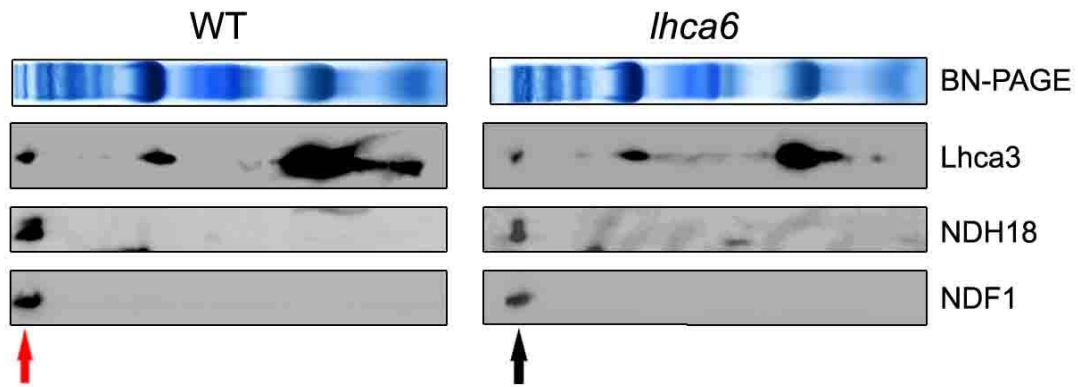
Supplemental Figure 4. Visible Phenotype of *lhca6* Mutant.

Seedlings were cultured at $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 4 weeks after germination. The leaves labeled 1–2 and 3–6 were used as immature and mature leaves, respectively, in Figures 4, 5, 7, and 8.



Supplemental Figure 5. NDH-PSI Supercomplex Content in *lhca5* and *lhca6*.

Thylakoids isolated from wild type (WT), *lhca5* and *lhca6* mutants were subjected to BN-PAGE analysis. After electrophoresis, the high-molecular-weight region corresponding to Band I was excised from the BN gel, and the proteins were denatured in the gel with SDS sample buffer. The proteins were further separated by 12.5% SDS-PAGE and immunodetected with specific antibodies against PsaA, NdhH and NdhL.



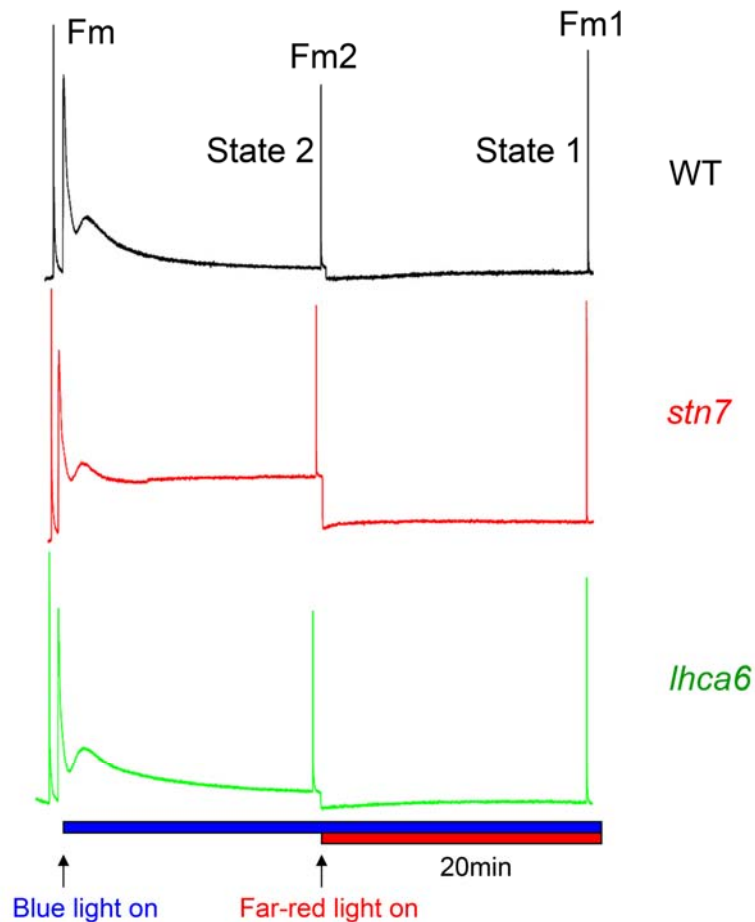
Supplemental Figure 6. Analysis of Thylakoid protein complexes from Wild-type (WT) and *lhca6* mature leaves.

Thylakoid membrane complexes separated by BN-PAGE were further subjected to 12.5% 2D SDS-PAGE, and the proteins were immunodetected with specific antibodies against Lhca3, NDH18 and NDF1. Positions of NDH-PSI supercomplex and putative smaller NDH-PSI supercomplex are indicated by red and black arrows respectively.



Supplemental Figure 7. Amino Acid Sequence Alignments of Lhca6 and Lhca2.

The sequences of At Lhca6 (At1g19150, Arabidopsis), Nt Lhca6 (DW000027, *Nicotiana tabacum*), Gm Lhca6 (EH258354, *Glycine max*), Zm Lhca6 (DV507315, *Zea mays*), Os Lhca6 (AK067780, *Oryza sativa*), At Lhca2 (At3g61470, Arabidopsis), and Os Lhca2 (AK104651, *Oryza sativa*) were aligned with CLUSTALW. Black boxes indicate strictly conserved amino acids, and gray boxes indicate closely related amino acids. The predicted cleavage sites for the plastid-targeting signal in At Lhca6 and At Lhca2 are indicated by black and clear arrows, respectively. The additional conserved N-terminal extension of the mature Lhca6 protein compared to Lhca2 is indicated as a bar above the sequences.



Supplemental Figure 8. State 1-State 2 transitions in Wild-type (WT), *stn7* and *lhca6* plants.

State transitions were investigated by measuring chlorophyll fluorescence as previous reports (Jensen et al., 2000; Bellafiore et al., 2005). The leaf was kept in the dark for 30 min and the maximal fluorescence yield (Fm) was measured with a saturating 0.8-s flash. Then the leaf was illuminated for 20 min with $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ blue light (which favors PSII) from high intensity light source equipped with a BLF-460B filter. The maximal fluorescence yield (Fm2) was then measured in this state (state 2). Subsequently the far-red light (which favors PSI) was switched on. After 15 min of illumination, the maximum fluorescence yield (Fm1) was measured in state 1.

Supplemental Table 1. r-Value between NDH Complex-Related Genes with *Lhca6*, *FKBP16-2* and *NDH18*.

Locus	<i>Lhca6</i>	<i>At4g39710</i>	<i>At5g43750</i>
<i>At1g70760: NdhL</i>	0.91	0.86	0.88
<i>At5g58260: NdhN</i>	0.92	0.87	0.85
<i>At1g74880: NdhO</i>	0.85	0.83	0.82
<i>At1g15980: NDF1/NDH48</i>	0.90	0.82	0.91
<i>At1g64770: NDF2/NDH45</i>	0.84	0.81	0.79
<i>At3g16250: NDF4</i>	0.93	0.90	0.89
<i>At1g18730: NDF6</i>	0.85	0.80	0.87
<i>At2g39470: PPL2</i>	0.93	0.89	0.87
<i>At5g52100: CRR1</i>	0.86	0.88	0.8
<i>At2g01590: CRR3</i>	0.88	0.83	0.81
<i>At1g19150: Lhca6</i>	-	0.89	0.88
<i>At4g39710: FKBP16-2</i>	0.89	-	0.83
<i>At5g43750: NDH18</i>	0.88	0.83	-

r-values were obtained from the ATTED-II database when *Lhca6*, *At4g39710*, *At5g43750* served as a query gene, respectively.

Supplemental Table 2. Primer List and Sequence

The following primers used for RNAi vectors construction:

Lhca6-Sence1:	5' AGTTCTAGACAATCAAAGATGACACTAGC 3'
Lhca6-Sence2:	5' AGTCTCGAGCAATCAAAGATGACACTAGC 3'
Lhca6-Antisence1:	5' CAGGGATCCATAAAGGACGGTCCGG 3'
Lhca6-Antisence2:	5' CAGGGTACCATAAAGGACGGTCCGG 3'
FKBP16-2-Sence1:	5' TACTCTAGATCAGGACTTGGATTTTGC 3'
FKBP16-2-Sence2:	5' TACCTCGAGTCAGGACTTGGATTTTGC 3'
FKBP16-2-Antisence1:	5' GGAGGATCCTTCATCTTGTATTACTTCCTG 3'
FKBP16-2-Antisence2:	5' GGAGGTACCTTCATCTTGTATTACTTCCTG 3'
NDH18-Sence1:	5' CGATTCTAGAAAACTCTGAGACAGAGCAAG 3'
NDH18-Sence2:	5' CGATGAATTCAAACTCTGAGACAGAGCAAG 3'
NDH18-Antisence1:	5' TACGGATCCTTATCGTGTGGCGATATCATC 3'

NDH18-Antisense2: 5' TCCGGTACCTTATCGTGTGGCGATATCATC 3'

The following primers used for RT-PCR:

Lhca6RT-LP: 5' AGAGAAGACCACATGTAGCGTCCAC 3'
Lhca6RT-RP: 5' GCAGAAGTTGTAACAACGGCGAGTG 3'
OsLhca6RT-LP: 5' TCACTCTAGACCATGGCTCTGCCCTC 3'
OsLhca6RT-RP: 5' AATCTCGAGAATGGGACGTGAATGCCGAG 3'
Lhca2RT-LP: 5' AGGTCAACAACGTCGAGAATAATGG 3'
Lhca2RT-RP: 5' GGAACCTTAATTTGCATTCGACCAC 3'
NDH18RT-LP: 5' ATGGCTCCAAAACCTCTGAGACAGAG 3'
NDH18RT-RP: 5' GTCATGATTATAACGAGATCACAATCC 3'
FKBP16-2RT-LP: 5' AAGGAGCTCGTTTTTCGGAGTGGGTTTAG 3'
FKBP16-2RT-RP: 5' TTGCTCGAGTCTTGTATTACTTCCTGGG 3'.
FKBP13RT-LP: 5' ATGAGCTCCTTGGGGTTTTTCAGTTG 3'
FKBP13RT-RP: 5' GCTCTCTCAAGCTTTACCTATGTACTC 3'
ACT-LP: 5' GAGAGATTCAGGTGCCCAG 3'
ACT-RP: 5' AGAGCGAGAGCGGGTTTTCA 3'

The following primers used for protein expression vectors construction:

NDH18antibody-LP1: 5' GGCGAATTCTTGAAGTTAGCGAAGCTG 3'
NDH18antibody-RP1: 5' ATCAAGCTTCATCCCAAGAGCTATAGC 3'
NDH18antibody-LP2: 5' TGTAAGCTTGAGATGGATAAACCTGACC 3'
NDH18antibody-RP2: 5' ATTCTCGAGTCGTGTGGCGATATCATC 3'
FKBP16-2antibody-LP: 5'- AAGGAGCTCGTTTTTCGGAGTGGGTTTAG -3'
FKBP16-2antibody-RP: 5'- TTGCTCGAGTCTTGTATTACTTCCTGGG -3'

REFERENCES ADDED FOR SUPPLEMENTAL INFORMATION

Bellafore, S., Barneche, F., Peltier, G., and Rochaix, J.-D. (2005). State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature* **433**: 892-895.

Jensen, P.E., Gilpin, M., Knoetzel, J., and Scheller, H.V. (2000). The PSI-K subunit of photosystem I is involved in the interaction between light-harvesting complex I and the photosystem I reaction center core. *J. Biol. Chem.* **275**: 24701–24708.