$NADP^+$ Supply Adjusts the Synthesis of Photosystem I in Arabidopsis Chloroplasts

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Supplemental Figure S1: Analysis of the transient increase in chlorophyll fluorescence after termination of AL illumination.

Supplemental Figure S2: P700 signals normalized to chlorophyll contents in WT and *nadk2-2* plants.

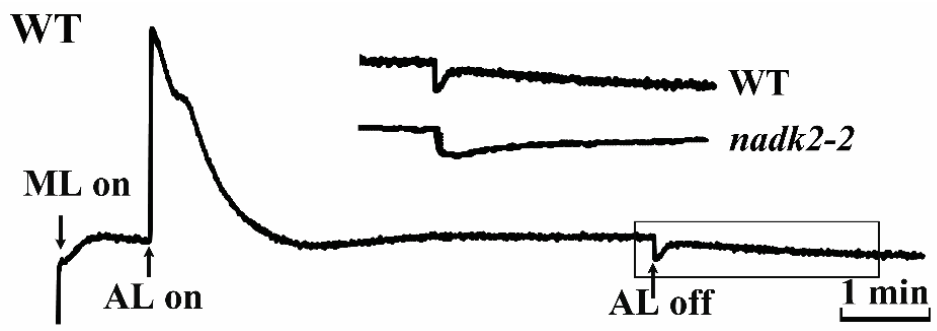
Supplemental Figure S3: CET rate in WT and *nadk2-2* plants.

Supplemental Figure S4: 2D BN/SDS-PAGE analysis of thylakoid proteins.

Supplemental Figure S5: Effect of the *NADK2* mutation on HCF145 activity and accumulation.

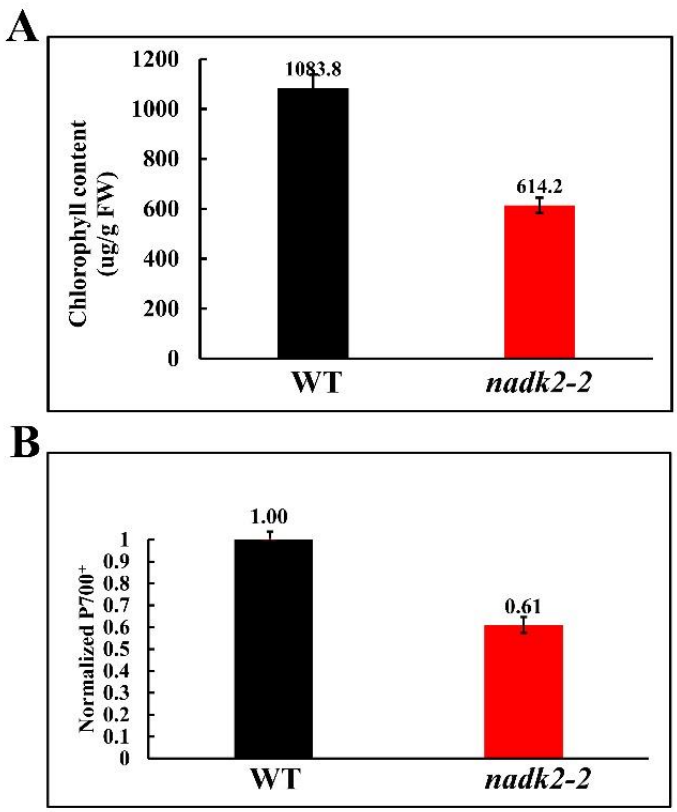
Supplemental Table S1: Photosynthetic parameters of WT and *nadk2-2*.

Supplemental Table S2: PCR primers used in this study.



Supplemental Figure S1. Analysis of the transient increase in chlorophyll fluorescence after termination of AL illumination.

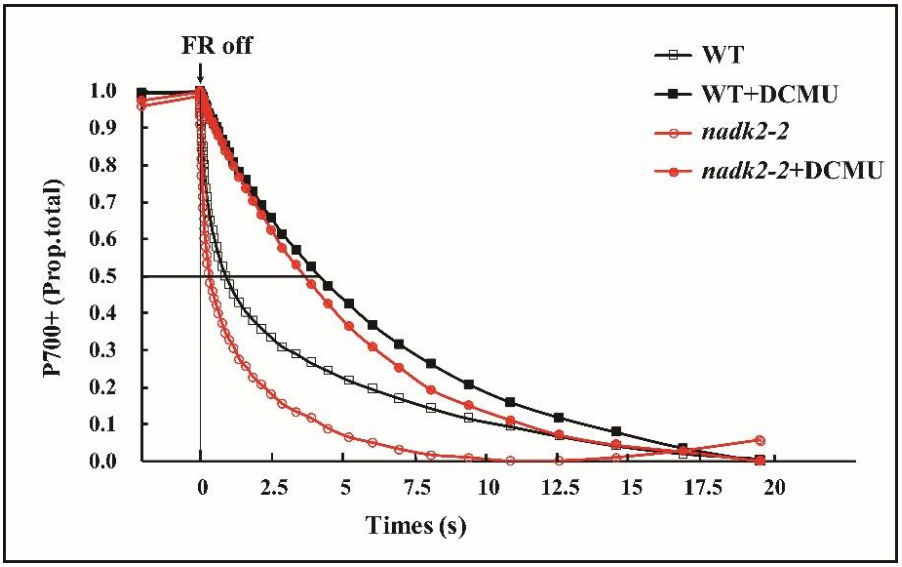
Chlorophyll fluorescence of Arabidopsis leaves was monitored using a pulse-amplitude-modulation chlorophyll fluorometer as descried (Shikanai et al. 1998). For WT and *nadk2-2* a bordered area is enlarged.



Supplemental Figure S2: P700 signals normalized to chlorophyll contents in WT and *nadk2-2* plants.

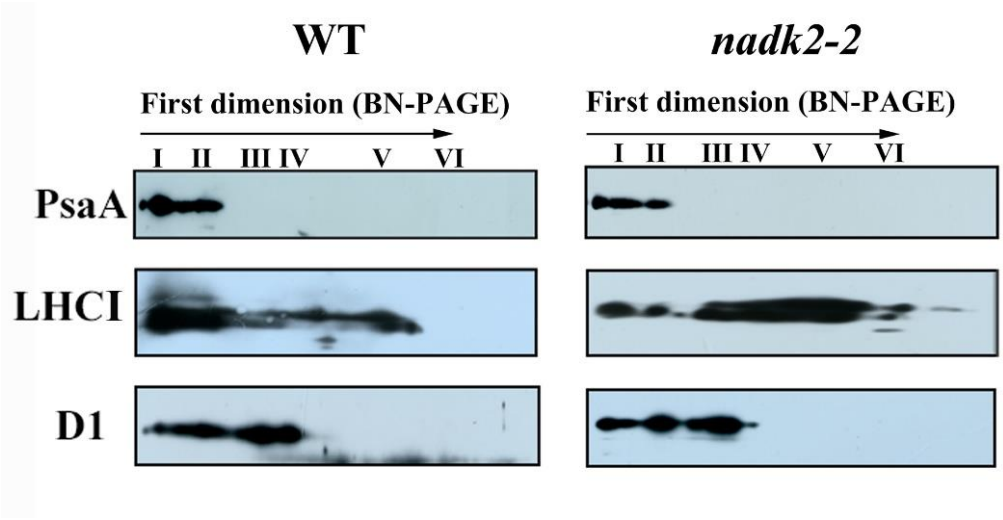
(A) Chlorophyll contents of three-week-old WT and *nadk2-2* plants. Values shown are means ± SD of six replicate experiments.

(B) Relative P700 signals normalized to chlorophyll contents in WT and *nadk2-2* plants. The P700 signal of WT was set to 1. Values shown are means ± SD of six replicate experiments.

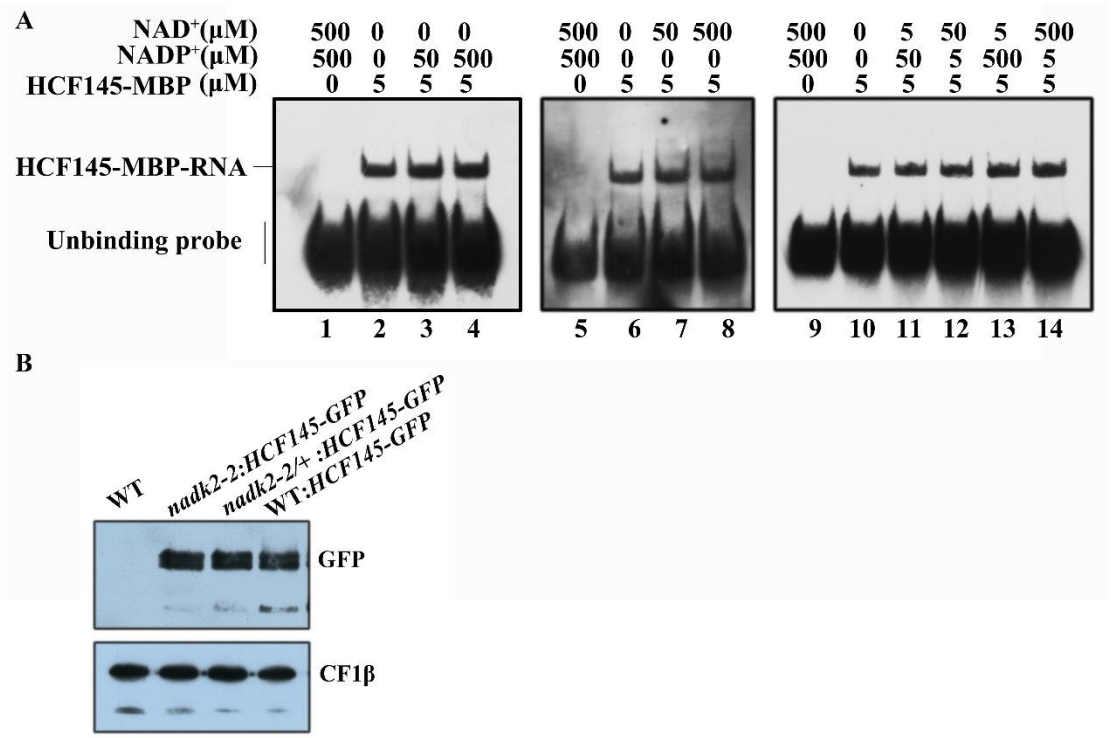


Supplemental Figure S3: CET rate in WT and *nadk2-2* plants.

The reduction of $P700^{+}$ following a 20 s flash of saturating far red (FR) light (~8,000 µmol m^{-2} s^{-1}) in leaves of WT and *nadk2-2* infiltrated with 20 µM DCMU was measured. To avoid osmotic effects, leaves were infiltrated in the presence of 300 mM sorbitol. The mean value of the half-time of P700 re-reduction (t1/2 P700 red) with or without DCMU treatment in WT and mutant plants were shown in Supplemental Table1.



Supplemental Figure S4: 2D BN/SDS-PAGE analysis of thylakoid proteins.
Thylakoid complexes were separated by BN-PAGE as shown in **Figure 4** and further subjected to SDS-PAGE. Gels were probed with antibodies against PsaA, LHCI, and D1.



Supplemental Figure S5: Effect of the *NADK2* mutation on HCF145 activity and accumulation.

(A) EMSA assay of RNA-binding of HCF145 in the presence of NAD^+ and $NADP^+$. The *psaA* 5′ UTR probe used was described previously (Manavski et al., 2015). The binding reaction contained the indicated concentrations of recombinant HCF145-MBP, NAD^+, and $NADP^+$.

(B) Accumulation of HCF145 proteins in homozygous *nadk2-2*, heterozygous *nadk2-2,* and WT plants. The full-length HCF145 protein C-terminally fused to GFP was introduced into a heterozygous *nadk2-2* line by the floral dip method. Homozygous and heterozygous *nadk2-2* lines and WT plants were selected by genotyping the resulting T2 population of the same T1 plant and the abundance of the HCF145-GFP protein was assayed via immunoblotting with the GFP antibody. WT plants were used as a negative control. A replicate gel probed with CF1β is shown below as loading control.

Supplemental Table S1. Photosynthetic parameters of WT and *nadk2-2*.

Parameter	Wild Type (n≥5[a])	*nadk2-2* (n≥5)
$t_{1/2}$ P700 red (ms)[b]	882 ± 42	307 ± 29
$t_{1/2}$ P700 red+DCMU(ms)[c]	4186 ±147	3612 ±118
NPQ	1.86± 0.04	3.24± 0.07
qE	1.54± 0.04	2.85± 0.07
qI	0.32± 0.04	0.39± 0.07

[a]Number of replicates

[b]Half-time of nonphotochemical P700 re-reduction in the dark (s) without DCMU treatment

[c]Half-time of nonphotochemical P700 re-reduction in the dark (s) with DCMU treatment

Supplemental Table S2. PCR primers used in this study.

Names	Primer sequence 5'→3'	Experiment
nadk2-1-LP	TATTGGGAACAGTGCCAACTC	T-DNA insertion
nadk2-1-RP	GAGAAATCCAAGAGACCCCAG	
SALKLBb1.3	ATTTTGCCGATTTCGGAAC	
S1	CGCCGGATGATGAGAGAAGT	Sequencing and RT-PCR
S2	TCTTTCGTGATCGGTCGTGT	
nadk coms	GCGCGGTACCATGTTCCTATGCTTTTGCCCTTGCC'	Complementation
nadk coma	GCGCGAGCTCTCATTTGTCATCGTCATCCTTGTAGTCTTTGTCATCGTCATCCTTGTAGTCTTTGTCATCGTCATCCTTGTAGTCTCAGAGAGCCTTTTGATCAAGACGCTCG3'	
Fw-BamHI-HCF145	GCGATATCGTCGACGGATCCAGCGGCGCCGGTGGTA	Recombinant HCF145-MBP
Rev-BamHI-HCF145	CCTGCAGGGAATTCGGATCCATATTGAACCCAATTG	
HCF145-1301-F ：	acgggggactctagaATGTCAGTGAGCAAGTTTCCAC	Complementation HCF145-FLAG
HCF145-1301-R ：	gtcatccttgtaatcATATTGAACCCAATTGATATCAAG	
Primers used to probe for northern blot and polysome analysis (forward / reverse)		
psbA	ACAACATTGTAGCTGCTCAC / CTAACACTAACGAATTATCC	
psbD	ATGACTATAGCCCTTGGTAA / AGAGCGTTTCCACGTGGTAG	
rps14	ATGGCAAAGAAAAGTTTGAT / TTACCAGCTTGATCTTGTTG	
psaA	CAATTGGCGCATTGGTCTTCGCAG/ / GTGCTCGCTGTTTCACCAGGGGCTG	
ycf3	CGTGGAGAACAGGCCATTCA / TTATTCGAAGCGCCTCGTGA	
psaC	CGATCCTATGTCACATTCAG / TCAATAAGCTAGACCCATAC	
23S rRNA	TTCAAACGAGGAAAGGCTTA / AGGAGAGCACTCATCTTGGG	
psaA	CAUAAUUGCUCUAGUGAAUAACUAAAGAAAAUAGAUGA/ AUAGAUGGAAGAUAGAAGAGA	