The chloroplast metalloproteases VAR2 and EGY1 act synergistically to regulate chloroplast development in Arabidopsis

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Running Title: *EGY1 regulates chloroplast development and proteostasis*

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Table S1. Primers used in this study.

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Figure S1

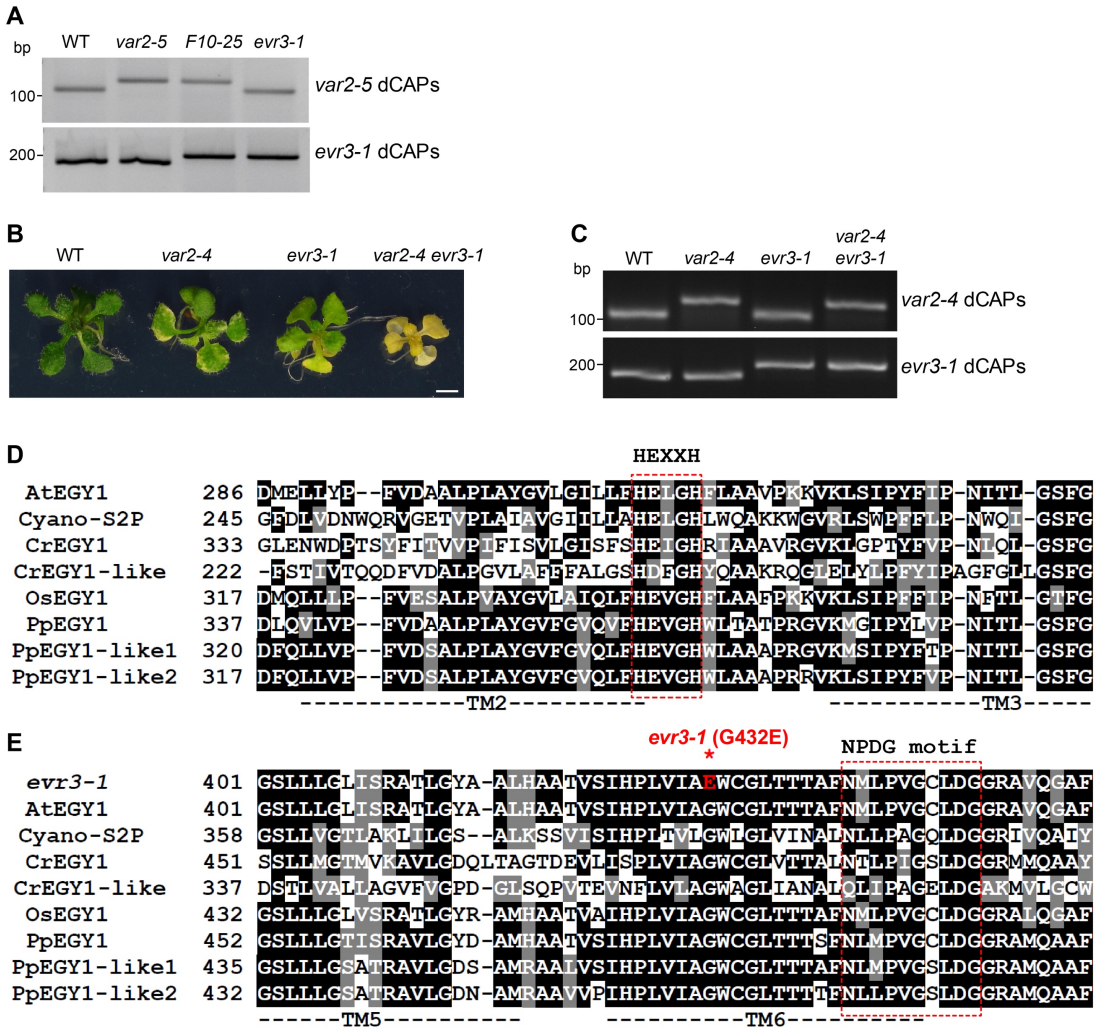

Figure S1. *evr3-1* is a new allele of *egy1*.

A. dCAPS genotyping of *VAR2* and *EVR3* loci for plants shown in Fig. 1A. B. Phenotypes of representative three-week-old WT, *var2-4*, *evr3-1* and *var2-4 evr3-1* grown on 1/2 MS medium supplemented with 1% sucrose. Bar, 5 mm. C. dCAPS genotyping of *VAR2* and *EVR3* loci for plants shown in B. D and E. Partial multiple sequence alignment of EGY1 and EGY1 homologues of representative photosynthetic species. Shown were the region containing the conserved zinc binding sites (HEXXD) (D) and the region containing the NPDG motif and the *evr3-1* mutation site (G432E) (E). Conserved motifs were framed with red boxes. Sequences of EGY1 homologues in *Synechocystis sp. PCC* 6714 (Cyano-S2P, WP_028947417.1), *Chlamydomonas reinhardtii* (CrEGY1, PNW86030.1; CrEGY1-like, PNW88890.1), *Oryza Sativa* (OsEGY1, XP_015630593.1), and *Physcomitrella patens* (PpEGY1, XP_024356580.1; PpEGY1-like1, XP_024359606.1; PpEGY1-like2, XP_024402167.1) were obtained from National Center for Biotechnology Information (NCBI).

Figure S2

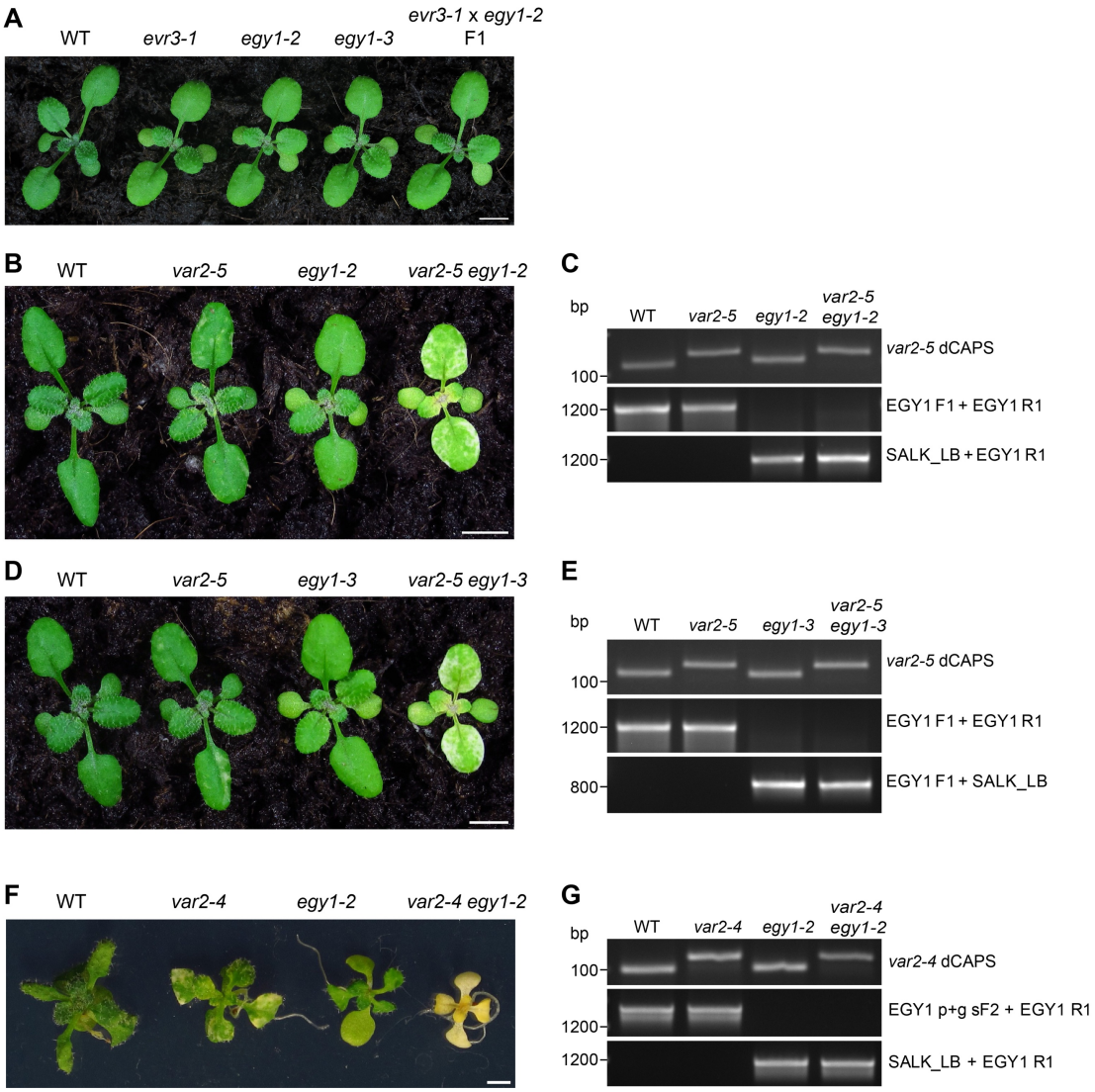

Figure S2. The enhancement of *var2* by *egy1-2* and *egy1-3*.

A. Phenotypes of representative two-week-old WT, *evr3-1*, *egy1-2*, *egy1-3*, and F1 of a cross between *evr3-1* and *egy1-2*. Bar, 5 mm. B. Phenotypes of representative two-week-old WT, *var2-5*, *egy1-2*, and *var2-5 egy1-2*. Bar, 5 mm. D. Phenotypes of representative two-week-old WT, *var2-5*, *egy1-3*, and *var2-5 egy1-3*. Bar, 5 mm. F. Phenotypes of representative three-week-old WT, *var2-4*, *egy1-2*, and *var2-4 egy1-2* grown on 1/2 MS medium supplemented with 1% sucrose. Bar, 5 mm. C, E, and G, PCR-based genotyping of plants shown in B, D, and F, respectively.

Figure S3

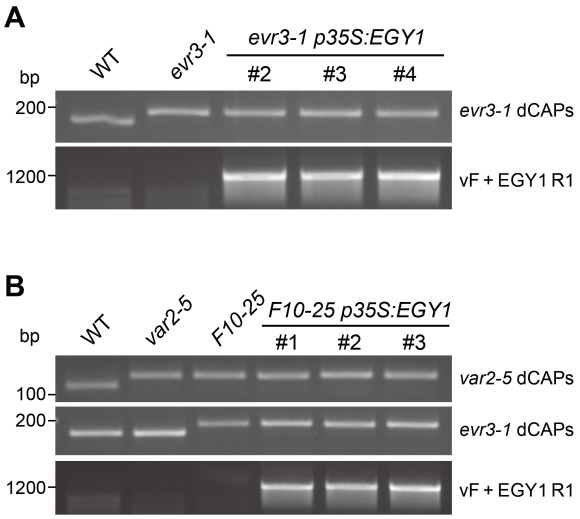

Figure S3. PCR based genotyping of *evr3-1* and *F10-25* complementation lines.

A. PCR-based genotyping of plants shown in Fig. 2C. B. PCR-based genotyping of plants shown in Fig. 2D.

Figure S4

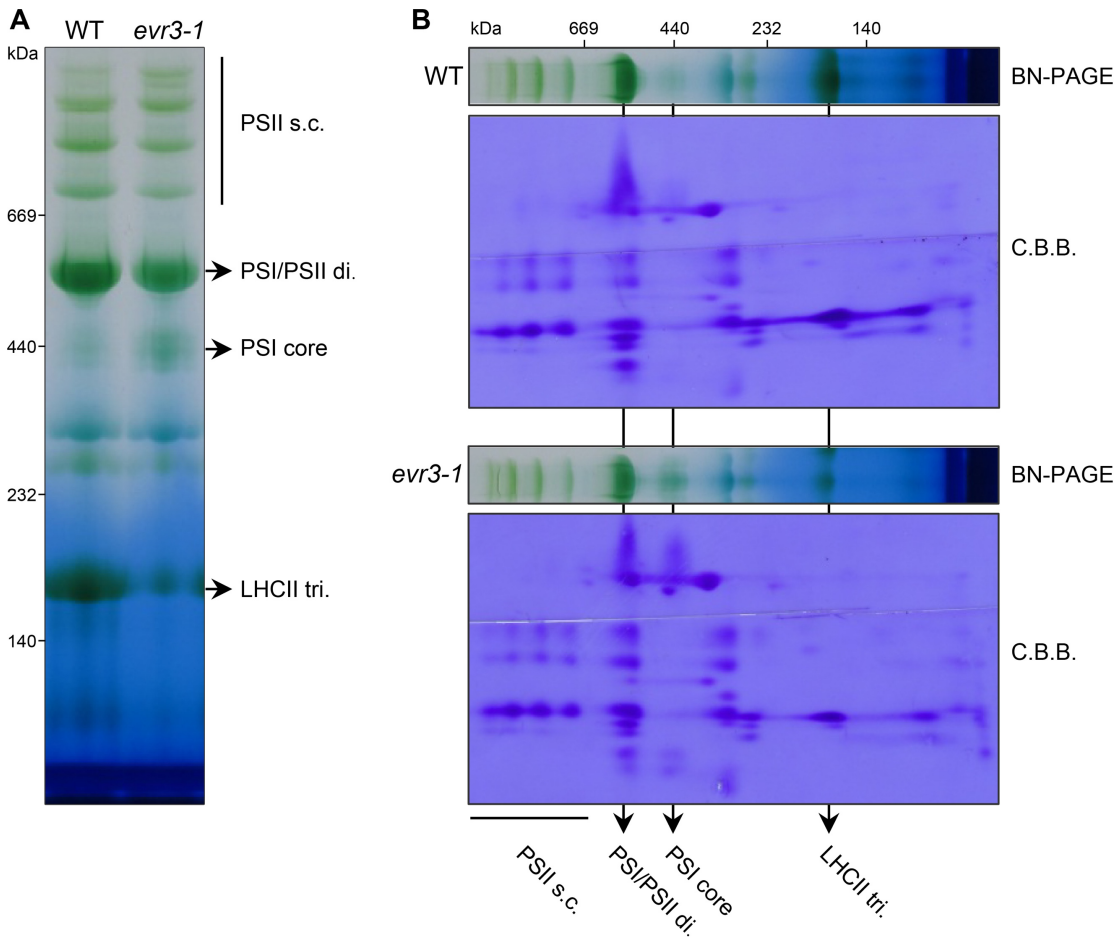

Figure S4. 2-D BN/SDS-Urea-PAGE analysis of photosynthetic complexes in WT and *evr3-1*. A. Thylakoid membranes from 4-week-old WT and *evr3-1* were solubilized with 1% β-dodecylmaltoside and resolved on 3%-12% BN-PAGE. B. 1-D BN-PAGE gel lanes shown in A were resolved on the 2-D SDS-PAGE and stained with Coomassie Brilliant Blue. Positions of PSII supercomplex (PSII s.c.), PSI/PSII dimer (PSI/PSII di.), PSI Core, and LHCII trimer (LHCII tri.) were marked.

Figure S5

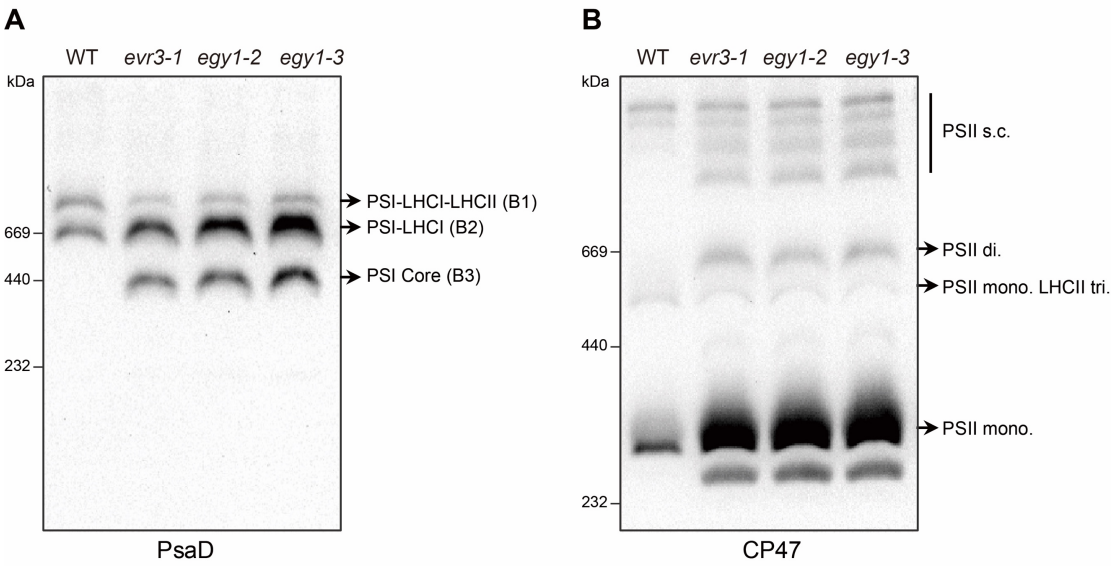

Figure S5. Abnormal accumulations of PSI and PSII complexes in three *egy1* mutants. Thylakoid membranes from 4-week-old WT, *evr3-1*, *egy1-2* and *egy1-3*, were solubilized with 2% digitonin, resolved by 3%-12% BN-PAGE, and probed with antibodies against PsaD to show PSI complexes (A), and against PsbB (CP47) to show PSII complexes (B).

Figure S6

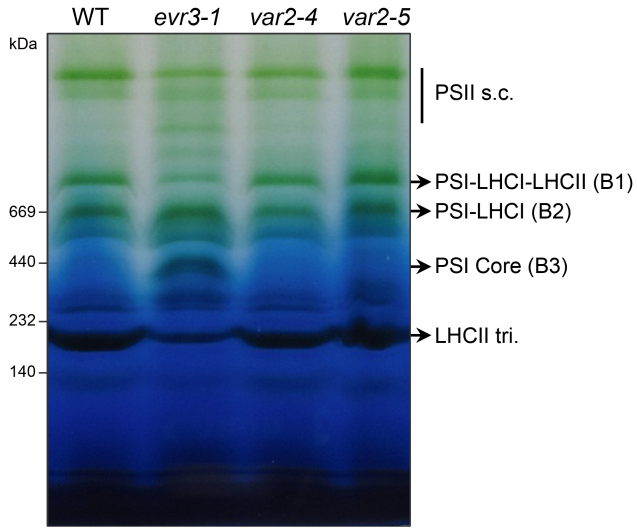

Figure S6. Accumulation of photosynthetic complexes in WT, *evr3-1*, *var2-4*, and *var2-5*. Thylakoid membranes from 4-week-old plants were solubilized with 2% digitonin and resolved on 3%-12% BN-PAGE. Positions of PSII supercomplex (PSII s.c.), PSI-LHCI-LHCII (B1), PSI-LHCI (B2), PSI Core (B3), and LHCII trimer (LHCII tri.) were marked.

Figure S7

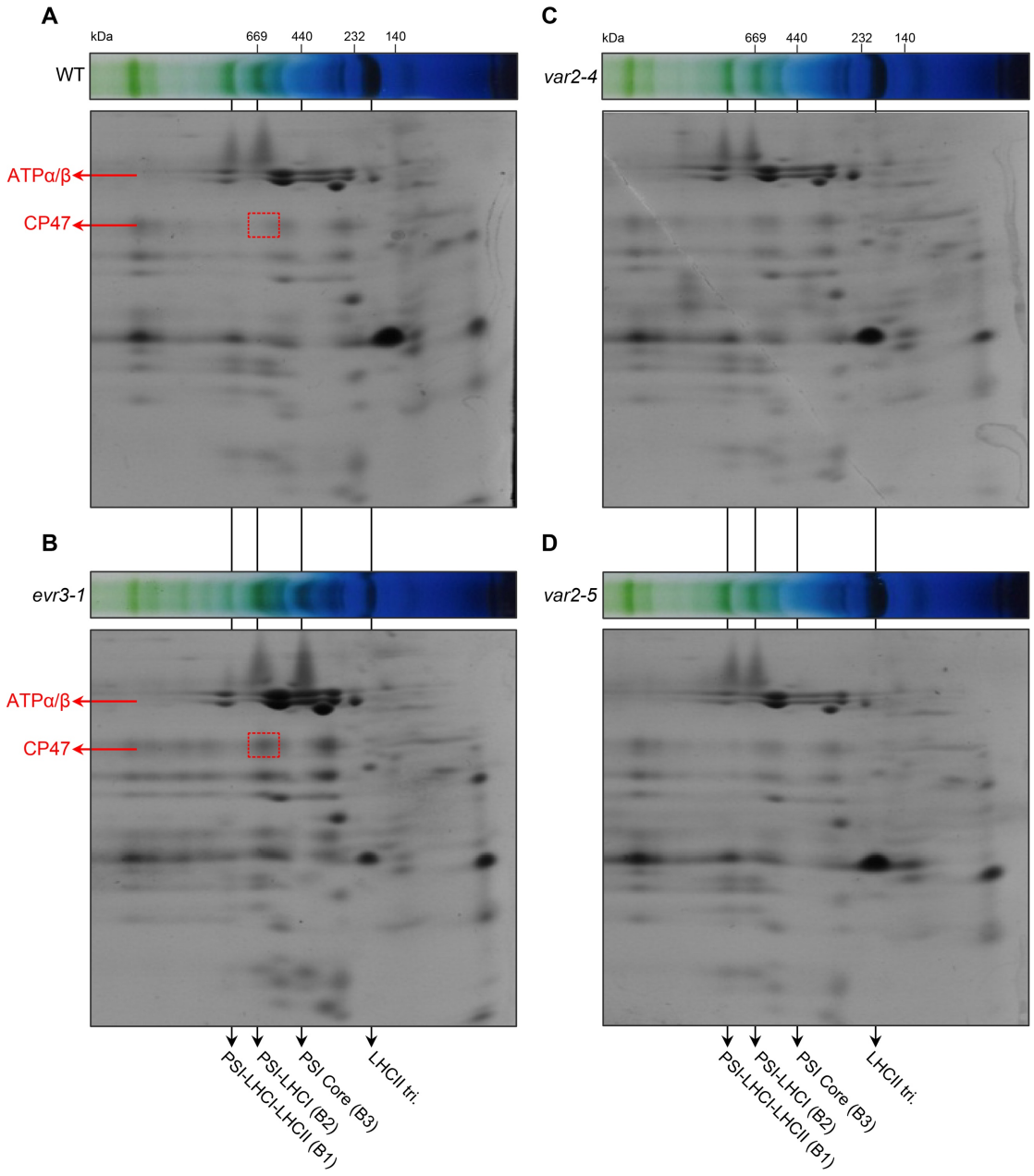

Figure S7. 2-D BN/SDS-Urea-PAGE analysis of photosynthetic complexes in WT, *evr3-1*, *var2-4*, and *var2-5*. Thylakoid membranes from 4-week-old WT (A), *evr3-1* (B), *var2-4* (C), and *var2-5* (D) were solubilized with 2% digitonin and resolved on 3%-12% BN-PAGE. BN-PAGE gel lanes were resolved on the 2-D SDS-PAGE and silver stained. Positions of PSI-LHCI-LHCII (B1), PSI-LHCI (B2), PSI Core (B3), and LHCII trimer (LHCII tri.) were marked. Red boxes indicated the positions of CP47 in PSII dimer. Note that the abundance of PSII dimer (represented by CP47) was different in WT and *evr3-1*.

Table S1. Primers used in this study.

Primer name	Primer sequences	Notes
F10-25 F	5’-AATCCACCCACTTGTAATTCCTG-3’	*evr3-1* dCAPS (cut WT sequence w/ EcoRII)
F10-25 R	5’-ATACAAGGAAGTACCTGTACAGC-3’	
VAR2-4 F	5’-GGCCAGGACGGTTTGACCTGCA-3’	*var2-4* dCAPS (cut WT sequence w/PstI)
VAR2-4 R	5’-TCAACACTTACCTGCACCAG-3’	
VAR2-5 F	5’-CAAGAAAGCCAAGGAGAAGGATC-3’	*var2-5* dCAPS (cut WT sequence w/BamHI)
VAR2-5 R	5’-TCTCAGTTAGGAGCTGATTG-3’	
EGY1 F1	5’-GTAACAGAGACGACAGTATC-3’	*egy1-2*, *egy1-3* genotyping
EGY1 R1	5’-GCAAGAAAGTGCCCTAATTC-3’	
EGY1 p+g sF2	5’-ACCAACATGACAGTCTGATC-3’	
SALK_LB	5’-GAACAACACTCAACCCTATCTC-3’	
VF	5’-CATGGATCCACTCTCGCTTTCTTCAT CATCTC-3’	genotyping *evr3-1* and *F10-25* complementation lines
AT5G35220 F	5’-CATGGATCCATGGGGACTCTCACCA GCGTC-3’	For *EGY1* cDNA cloning
AT5G35220 R	5’-CATGGATCCTCAAAATGTGGTTACAA GCCCTATG-3’	

Table S2. Antibodies used in this study.

Proteins	Antibodies (Catalog Number)
PsbA	Agrisera (AS05 084)
PsbB	PhytoAB (PHY0058A)
PsbC	PhytoAB (PHY0059A)
PsbD	PhytoAB (PHY0060)
PsaA	PhytoAB (PHY0342)
PsaC	PhytoAB (PHY0055A)
PsaD	PhytoAB (PHY0343)
PsaF	Kindly provided by Dr. Aigen Fu
Cytf	Kindly provided by Dr. Aigen Fu
PetC	Agrisera (AS08 330)
LhcA1	PhytoAB (PHY0043S)
LhcA2	PhytoAB (PHY0082S)
LhcB2	Agrisera (AS01 003)
VAR2/FtsH2	Qi et al., 2016