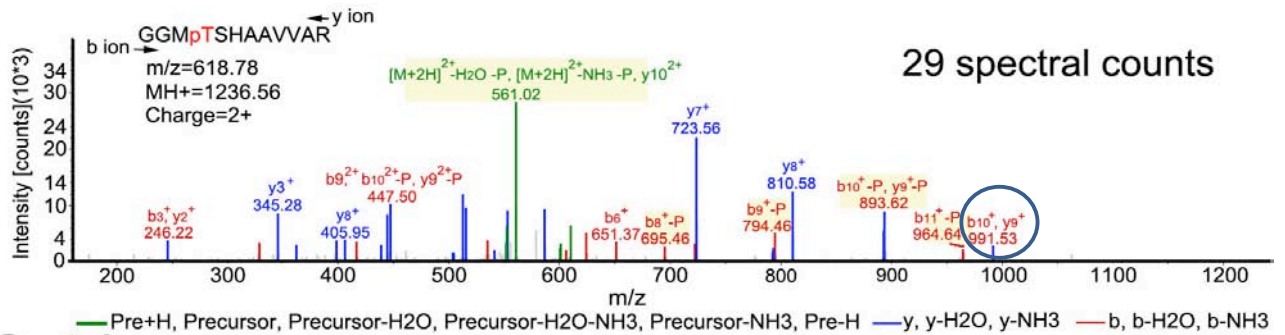
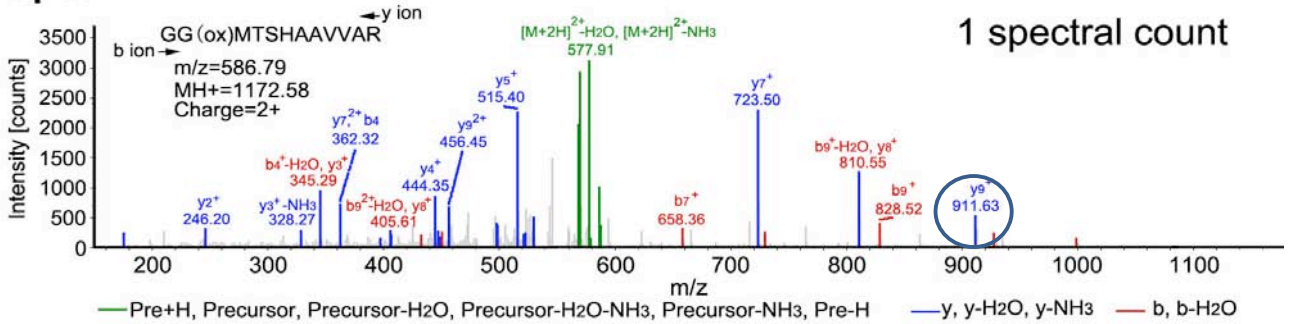


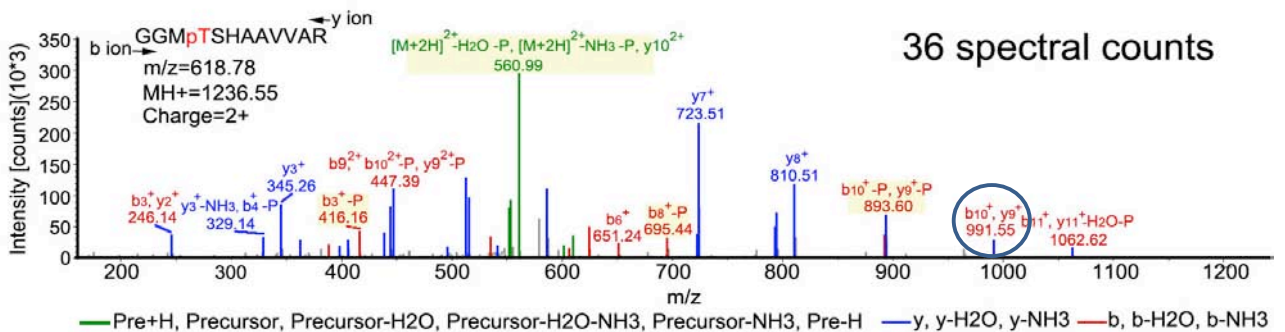
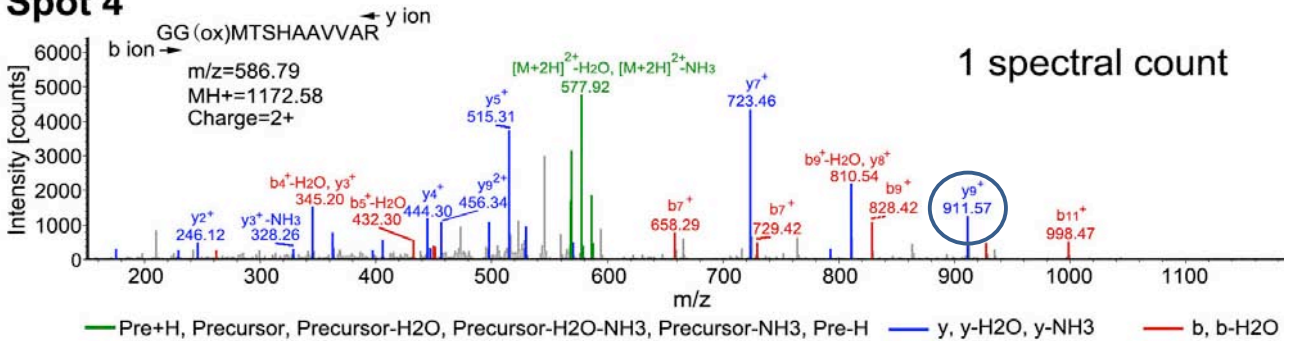
Supplemental Data

This article contains Supplemental Fig. S1–12 and Table S1.

Spot 2



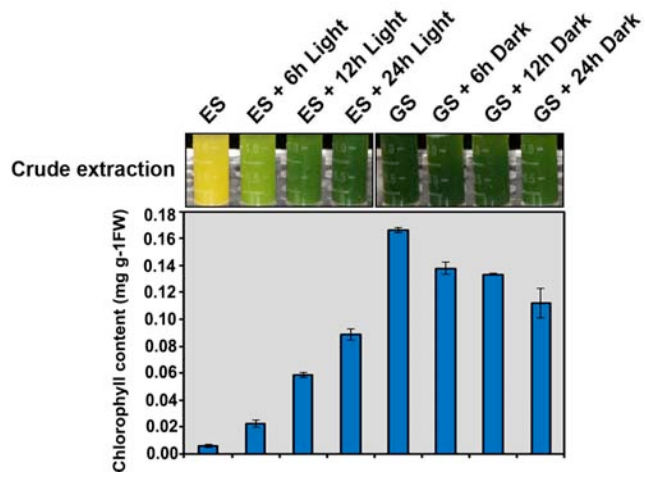
Spot 4



Supplemental Figure S1.

Representative high-resolution MS/MS ion spectra of the unphosphorylated and phosphorylated peptide GGMTSHAAVVAR (residues 524 to 535) identified in spots 2 and 4. Ions corresponding to y and b fragments of the GGMTSHAAVVAR sequence are labeled. The y9 ion (circled) from the phospho and non-phosphopeptides differed by 80 Da owing to phosphorylation on Thr527. A total of 29 and 36 phospho-spectra (m/z 618.78) were acquired, whereas the non-phosphopeptide (m/z 586.79) spectra were only identified once each in spots 2 and 4.

Chen et al. Supplemental Fig.S2

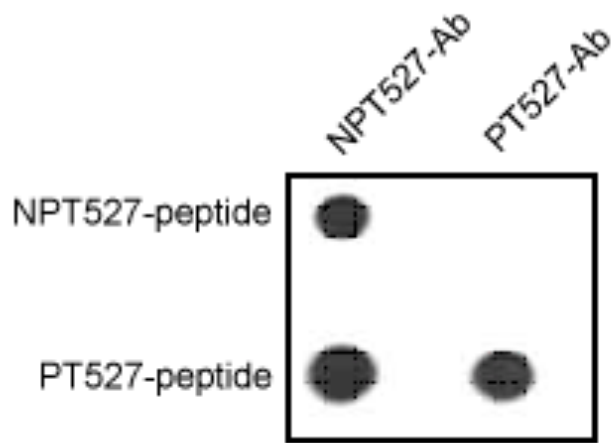


Supplemental Figure S2.

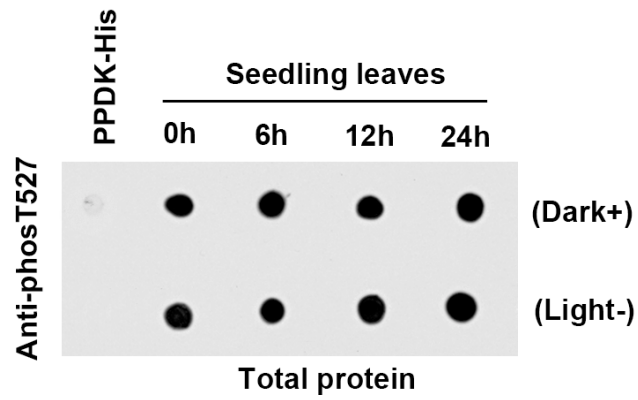
The crude extracts and chlorophyll content of maize leaves grown under different illumination regimens.

Chen et al. Supplemental Fig.S3

A



B

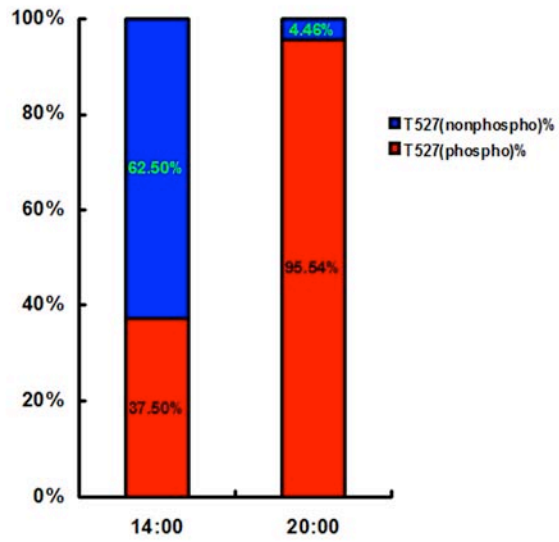
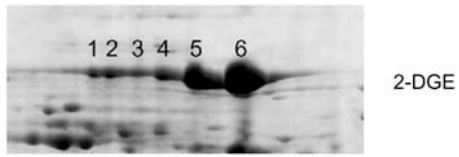


Supplemental Figure S3.

Assessment of specificity of the anti-phospho-T527 antibody.

(A) The phospho-antibody for Thr527 specifically recognizes phosphopeptide GGM(p)TSHAAVVAR (residues 524-535), whereas the antibody generated by the non-phosphorylated peptide GGMTSHAAVVAR (residues 524-535) cross-reacts with both peptide and the non-phosphopeptide antigen. NP, non-phosphorylated; P, phosphorylated; Ab, antibody. (B) The antibody specifically recognized phosThr527-PPDK from soluble leaf extracts of different illumination scenarios, whereas it did not cross-react with the recombinant PPDK protein raised by prokaryotic expression (non-phosphoisoform).

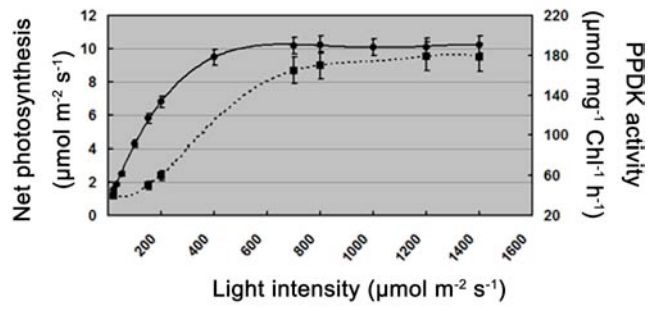
Chen et al. Supplemental Fig.S4



Supplemental Figure S4.

The numbers of MS/MS spectra counts for phosphopeptides and unphosphorylated peptides containing Thr527. Each of the six spots was excised from 2DGE gels of the maize leaves harvested at 14:00 ($1400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), 20:00 ($0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) respectively and digested with trypsin. The tryptic peptides from the six protein spots excised from each 2DGE gels combined together and then directly subjected to MS analysis without phosphopeptide enrichment. The numbers of MS/MS spectral counts of the peptides containing nonphospho- and phospho-Thr527 were listed. The histogram showed the percentages of nonphospho- and phospho-Thr527.

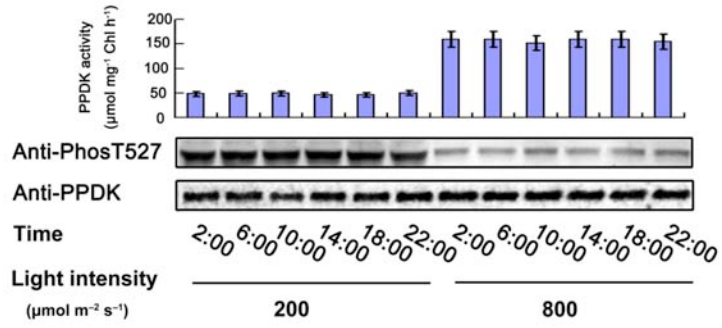
Chen et al. Supplemental Fig.S5



Supplemental Figure S5.

Correlations between net photosynthesis rates and PPDK activity in maize leaves grown under different levels of light intensity. The square and circle dashed lines represent PPDK activity and net photosynthesis rates, respectively.

Chen et al. Supplemental Fig.S6



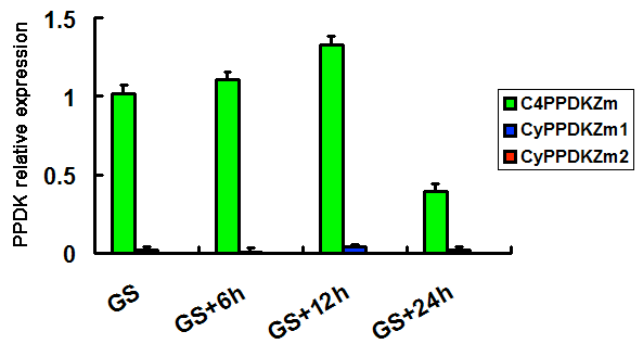
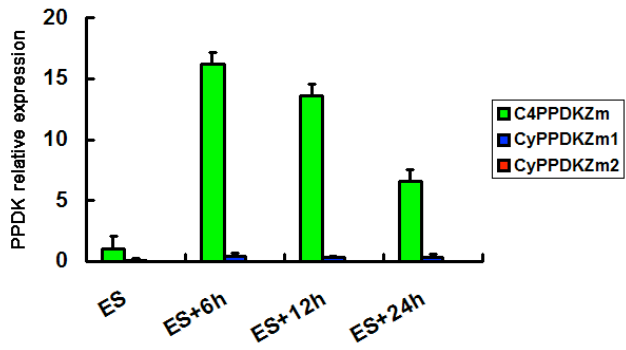
Supplemental Figure S6.

PPDK activity and the phosphorylation of Thr527 in PPDK are independent of circadian clock.

PPDK activities and the phosphorylation levels were assayed in the maize seedlings illuminated with light intensities of 200 and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, and harvested every 4 hours.

PPDK was used as a loading control. Antibodies specific against phosphorylated Thr527 (PhosT527) and PPDK were used in the western blotting analysis.

Chen et al. Supplemental Fig.S7

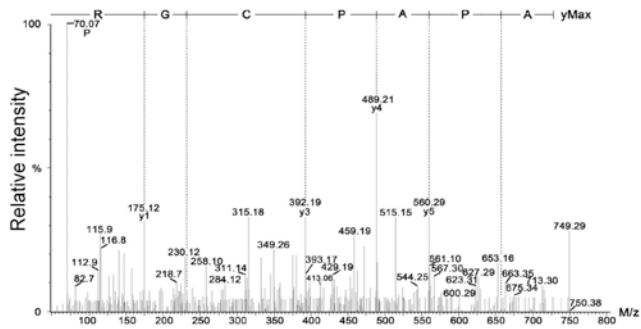
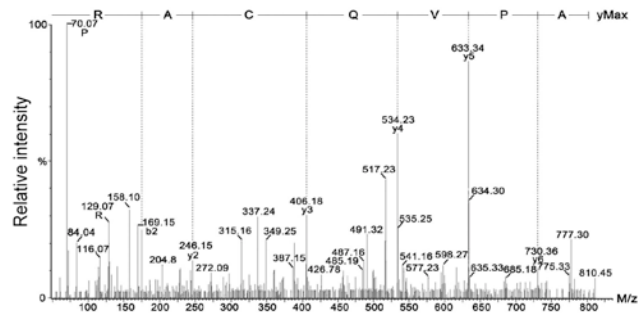


Supplemental Figure S7.

Expression profiling of *C₄ppdk* and the 2 types of *C₃ppdk* in maize leaves under different illumination conditions.

(A) Relative mRNA levels of the three genes in ES exposed to light conditions and (B) GS exposed to dark condition. Relative amounts of mRNA were normalized to the house keeping genes, actin and tubulin. Bars represent the SD.

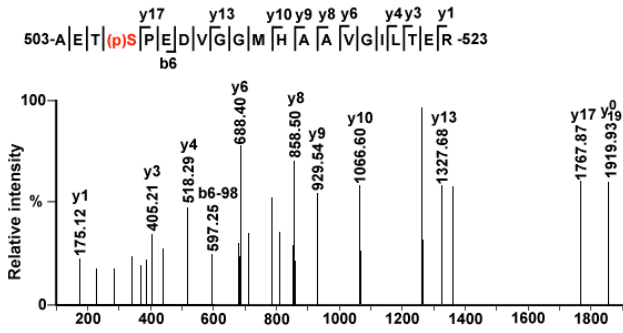
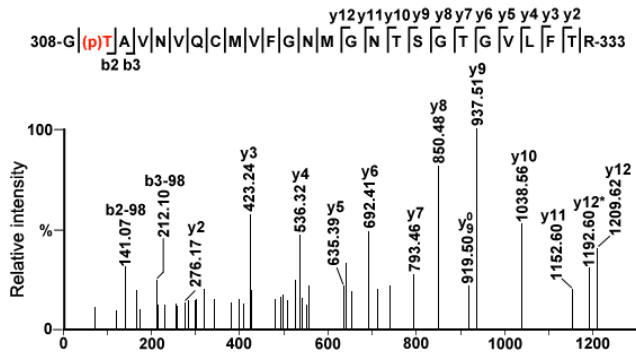
Chen et al. Supplemental Fig.S8

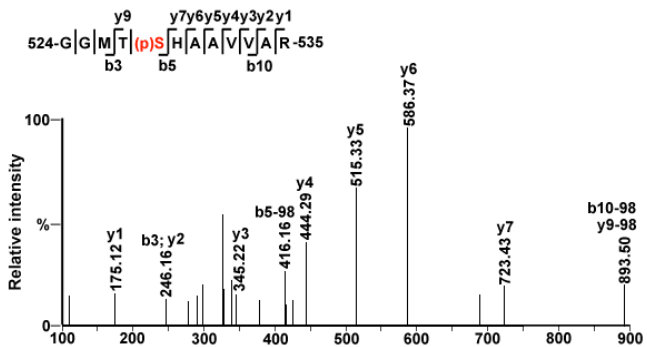
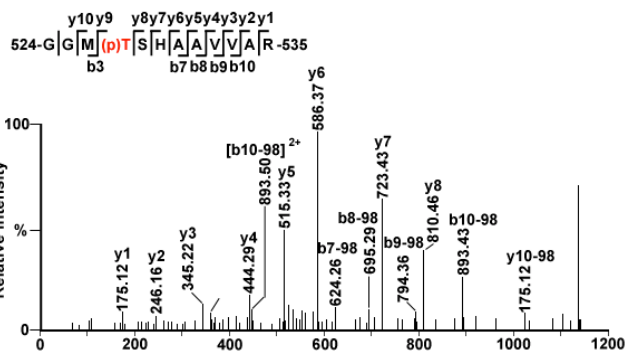


Supplemental Figure S8.

MS/MS spectra reveal the structure of N termini of mature CyPPDKZm1 and CyPPDKZm2.

Chen et al. Supplemental Fig.S9

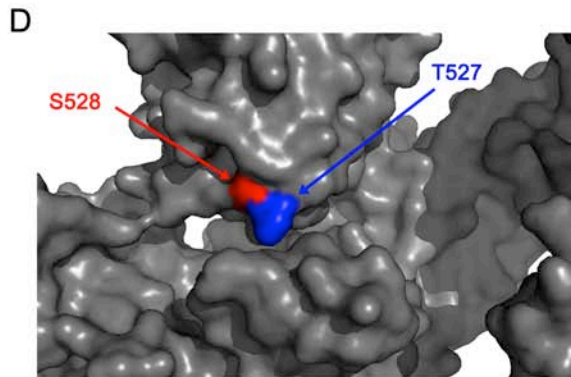
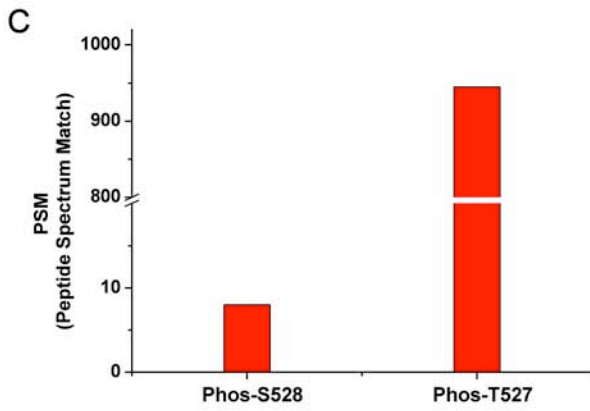
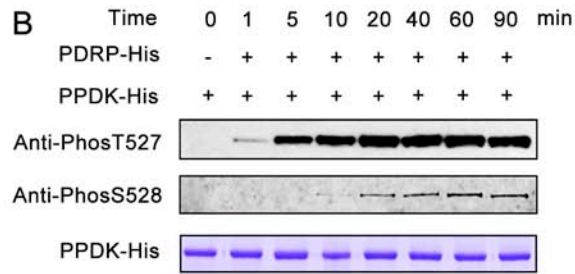
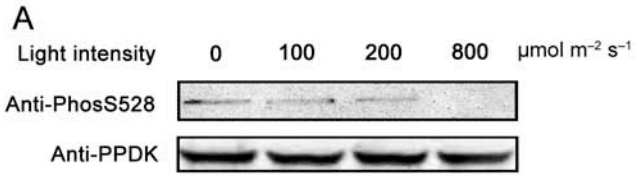




Supplemental Figure S9.

MS/MS spectra of the identified phosphorylated peptides in maize C₄PPDK. MS spectra of 4 phosphopeptides containing the phosphorylation sites Thr309, Ser506, Thr527, and Ser528: (A) 308-G(p)TAVNVQCMVFGNMGNTSGTGVLFR-338, (B) 503-AET(p)SPEDVGGMHAAVGILTER-523, (C) 524-GGM(p)TSHAAVVAR-535, and (D) 524-GGMT(p)SHAAVVAR-535.

Chen et al. Supplemental Fig.S10



Supplemental Fig.S10

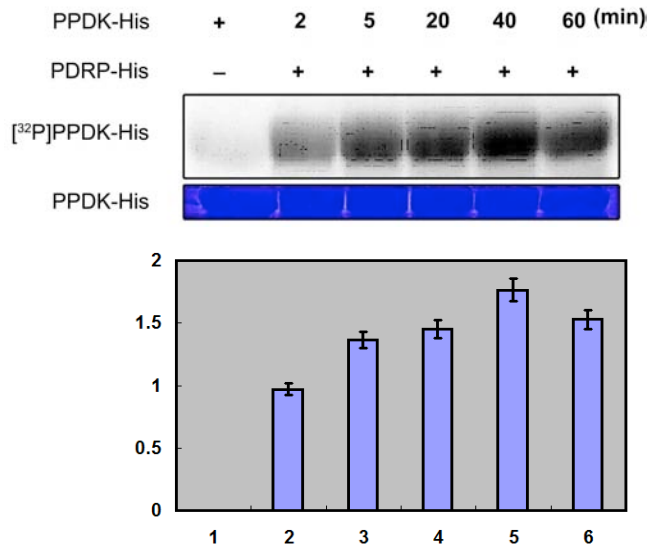
(A) The phosphorylation levels of PPDK at Ser528 in maize seedlings illuminated with different light intensities. An antibody specific to phosphorylated Ser528 of PPDK was used to detect the level of phosphorylation in maize seedlings illuminated with light intensities of 0, 100, 200, and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 30 min. PPDK was used as loading control.

(B) Time courses of PDRP catalyzing the phosphorylation of PPDK at Thr527 and Ser528 *in vitro*. Antibodies specific to Thr528 and Ser527 were used to analyze the phosphorylation levels of Thr527 and Ser528 at the different time points. The loading control was the ~95 kDa His-C₄PPDK protein, as revealed by Coomassie blue staining of the same gel.

(C) The number of MS/MS spectra counts of phosphopeptides containing Ser528. Each of the six spots was excised from 2DGE gels of proteins extracted from maize leaves that were illuminated for 30 min with light intensities of 0 and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and then digested with trypsin. The tryptic peptides were used in the MS analysis after phosphopeptide enrichment.

(D) Structural analysis of the side chains of Thr527 and Ser528 in maize PPDK (pdb ID: 1vbg). The blue and red areas represent the surface areas of Thr527 and Ser528, respectively, as calculated by the PISA server (website: http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html).

Chen et al. Supplemental Fig.S11

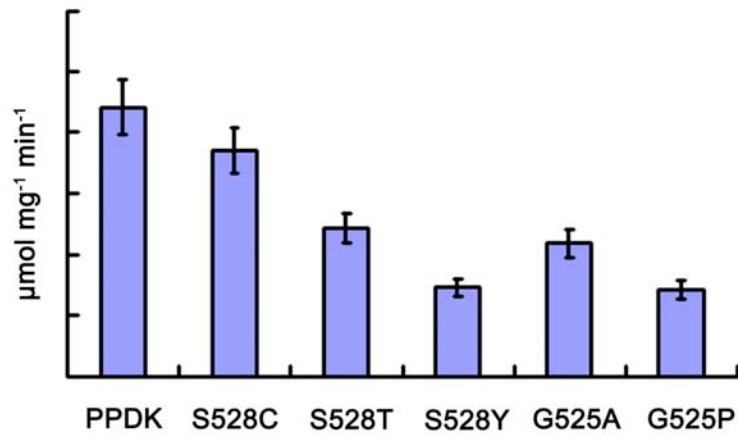


Supplemental Figure S11.

A time course of phosphorylation of PPDK by PDRP.

Autoradio graphic analysis of the phosphorylation reaction of recombinant C₄PPDK and PDRP by [γ -³²P] ADP. The loading control was the ~95 kDa His-C₄PPDK protein as revealed by Coomassie blue staining of the same gel.

Chen et al. Supplemental Fig.S12



Supplemental Fig.S12

Analysis of the enzyme activities of recombinant C₄PPDK and its site-directed mutant proteins.

Supplemental Table S1. Primers for PPDK mutagenesis

T309A	5'-gcctcagggcgccgccgtgaac-3' 5'-gttcacggcgcccccctgaggc-3'
S506A	5'-taagggcggagaccgccctgaggacgttg-3' 5'-caacgtcctcagggcggtctccgccctta-3'
T527A	5'-gaggggtggcatggctcccacgctgc-3' 5'-gcagcgtgggaagccatgccaccctc-3'
T527D	5'-gagaggggtggcatggattcccacgctgctg-3' 5'-acagcagcgtgggaatccatgccaccctctc-3'
S528A	5'-agaggggtggcatgactcccacgctg-3' 5'-cagcgtggcagtcagccaccctct-3'
H529A	5'-gtggcatgactccgccgctgctgtggtcg-3' 5'-cgaccacagcagcggcggaagtcagccac-3'
S528T	5'-agaggggtggcatgactaccacgctg-3' 5'-cagcgtgggtagtcagccaccctct-3'
S528C	5'-gggtggcatgactgccacgctgctg-3' 5'-cagcagcgtggcaagtcagccacc-3'
S528Y	5'-acagagaggggtggcatgacttatcacgctgctg-3' 5'-cacagcagcgtgataagtcagccaccctctctgt-3'
S528D	5'-acagagaggggtggcatgactgaccacgctgctg-3' 5'-cagcagcgtggtcagtcagccaccctctctgt-3'
G525A	5'-tacagagaggggtccatgacttcccacg-3' 5'-cgtgggaagtcagccaccctctctgta-3'
G525P	5'-cttacagagaggggtccatgacttcccacgc-3' 5'-gcgtgggaagtcagggaccctctctgtaag-3'
