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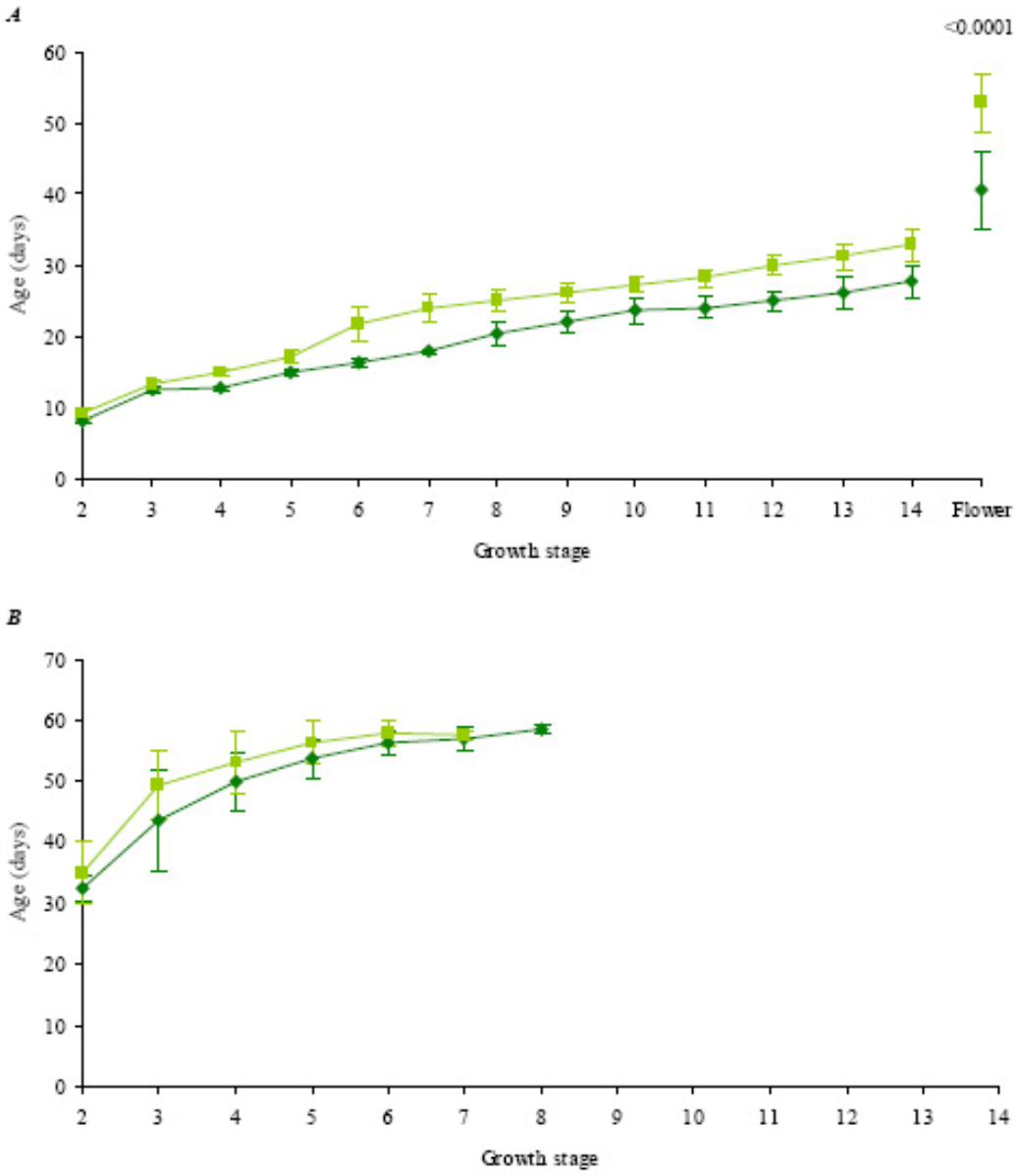

Fig. S1. Comparison of wild-type and *alx13* developmental rates under LL or vLL growth conditions.

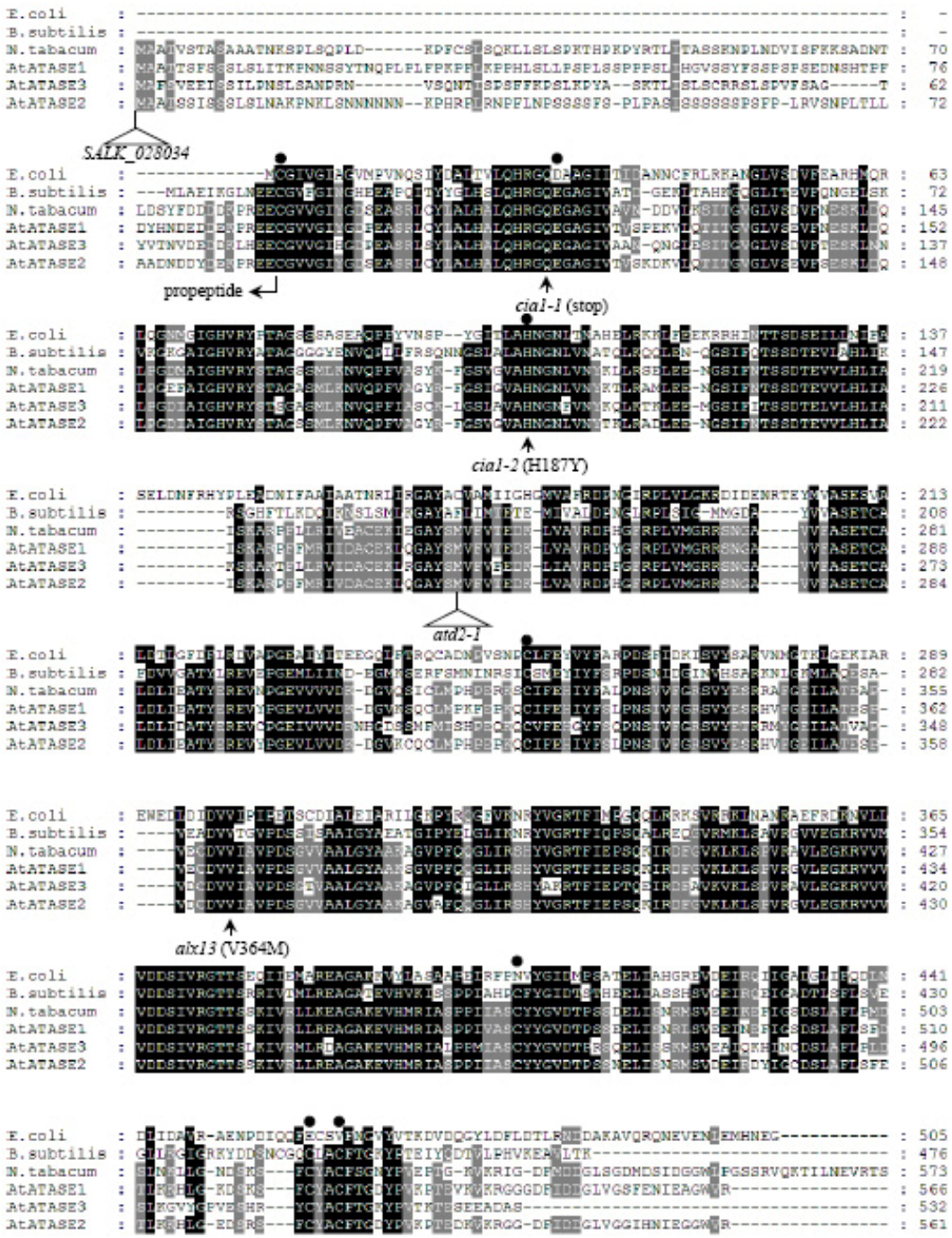

Fig. S2. Protein sequence alignments of the *Arabidopsis* ATASEs and homologues from three other species.

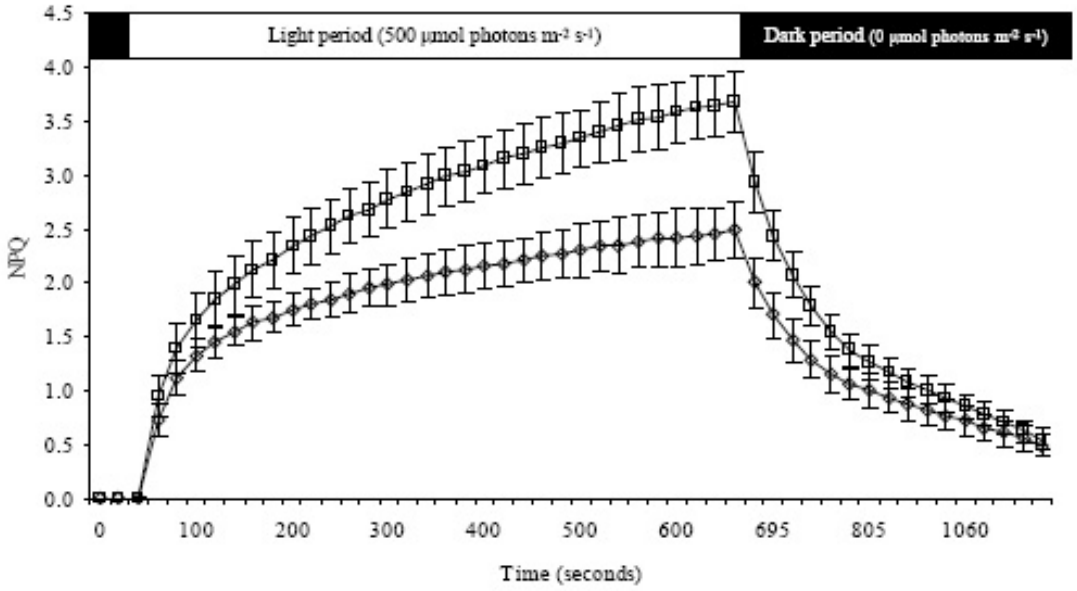

Fig. S3. Non-photochemical quenching induction and relaxation kinetics of *alx13*.

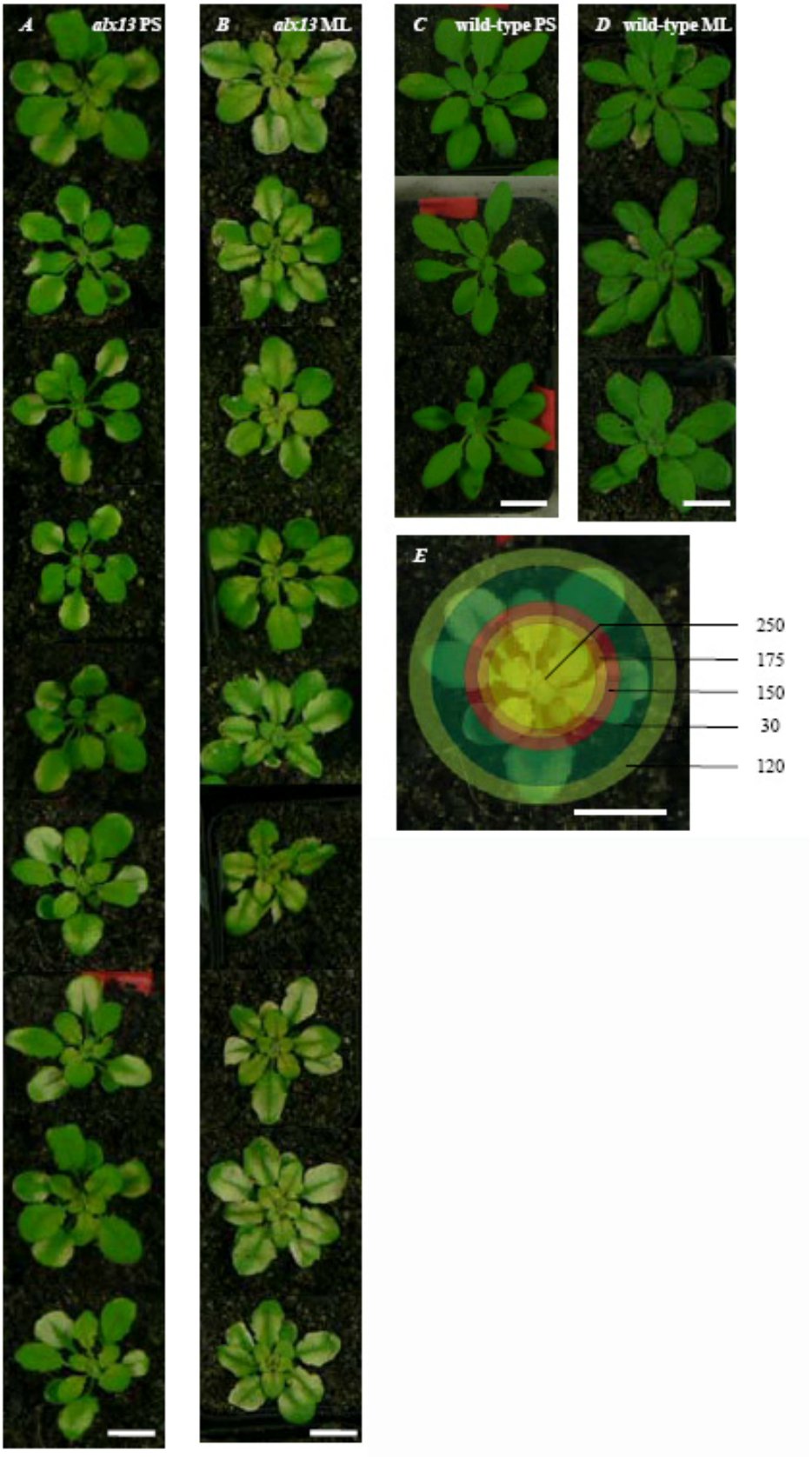

Fig. S4. Analysis of *alx13* plants grown under partial shade.

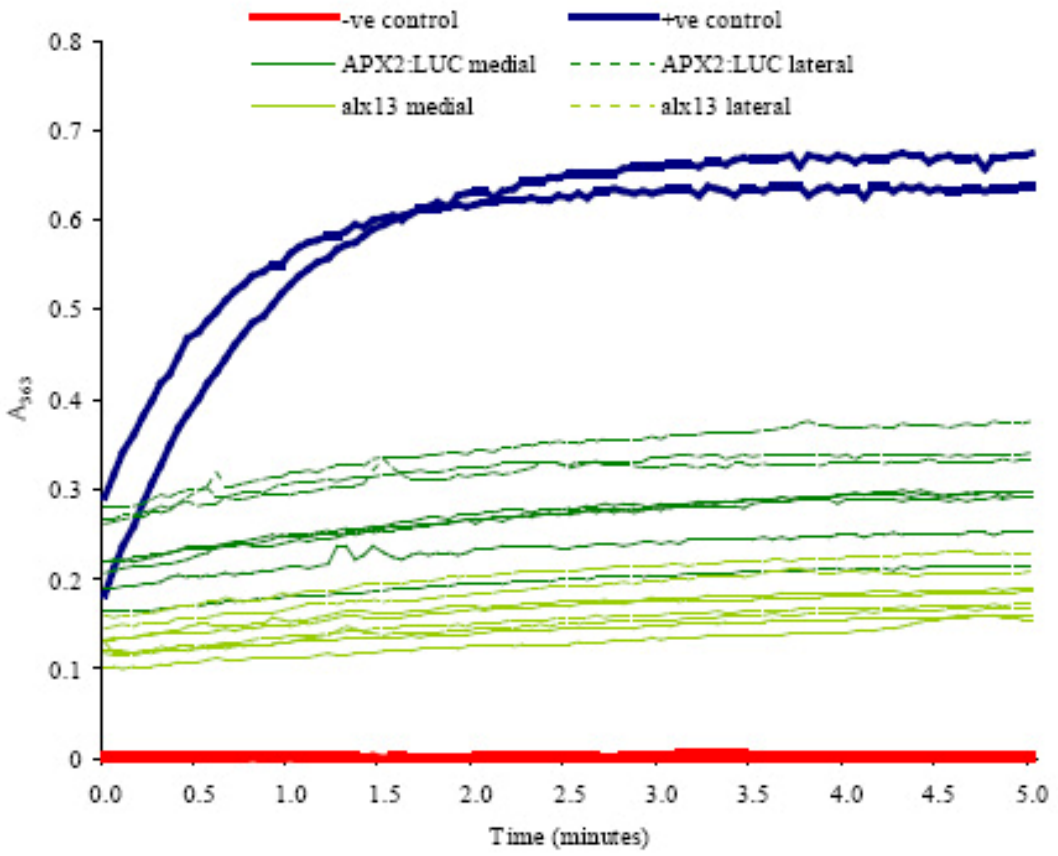

Fig. S5. Example glutamate dehydrogenase activity assay for determination of ATASE enzyme activity in *APX2:LUC* and *alx13* tissue sectors.

Protocol S1. Procedure for the determination of ATASE enzyme activity in *Arabidopsis*.

ATASE activity can be determined by measuring the production of either glutamate (Glu) or phosphoribosylamine (PRA) from the enzyme-catalyzed reaction of glutamine (Gln) and 5-phosphoribosyl-1-pyrophosphate (PRPP). As shown below, every molecule of PRPP to be converted to PRA requires deamination of one molecule of Gln to Glu (Mei and Zalkin, 1989; Wyngaarden and Ashton, 1959):

$$PRPP + Gln + H_2O \rightarrow PRA + Glu + pyrophosphate$$

In addition to its catalysis of this reaction, ATASE can also mediate the hydrolysis of Gln in the absence of PRPP, albeit at a much reduced rate. However, at Gln of <20 mM this PRPP-independent ATASE activity is insignificant. At such concentrations, the rate of PRA production can more or less be correlated directly with the rate of PRPP-dependent Glu production (Kim *et al.*, 1996). As such, Glu accumulation has long been utilized as a measure of ATASE activity, as an alternative to more difficult direct assessments of PRPP consumption or PRA production. Glu levels are estimated by carrying out a reaction with glutamate dehydrogenase (GDH), which mediates the oxidative deamination of Glu to α-ketoglutarate (Wyngaarden and Ashton, 1959):

$$Glu + H_2O + X \leftrightarrow \alpha\text{-ketoglutarate} + NH_4^+ + XH$$

In this reaction X represents a proton acceptor which is reduced during the oxidative deamination of glutamate. An oxidizing agent commonly used for this purpose is 3-acetylpyridine adenine dinucleotide ($APAD^+$), an analogue of NAD^+ and $NADP^+$. The production of the reduced form of $APAD^+$, APADH, is monitored as the deamination reaction proceeds as an increase in absorbance at 363 nm (Shiio and Ishii, 1969).

Three solutions are required for the assessment of ATASE activity: an extraction buffer, into which proteins are separated and ATASEs are stabilized; an ATASE reaction buffer, in which the conversion of PRPP to PRA via deamination of Gln is performed; and a glutamate dehydrogenase reaction buffer, in which the oxidative

deamination of Glu to α-ketoglutarate may be carried out and observed spectrophotometrically. As each solution contains components that are prohibitively expensive or may be unstable if stored in dissolved state, partial buffers were prepared and autoclave sterilized, with the more sensitive compounds added at the time that the experiment was to be performed. The complete components of each buffer solution are described below, as well as detailed instructions for the preparation of the partial buffer solutions and the amounts of labile components which should be added only at the time of the assay.

The complete ATASE extraction buffer consisted of the following: 100 mM HEPES/KOH pH 6.8, 1 mM EDTA, 1 mM $MgCl_2$, 5 mM L-glutathione, 20 mM DL-DTT and 0.1 mM PEFABLOC SC. 200 mL of partial ATASE extraction buffer was prepared by dissolving 4766 mg HEPES, 74.5 mg EDTA and 40.7 mg $MgCl_2.6H_2O$ in 200 mL H_2O. The solution was made to pH 6.8 using 10 M KOH; a neutral or acidic pH is necessary to ensure that glutamine amidotransferase activity is favoured over direct binding of NH_3 and consequent Gln-independent amination (Messenger and Zalkin, 1979). To complete the buffer, 0.768 mg rxn^{-1} L-GSH, 1.54 mg rxn^{-1} DL-DTT and 5 µL rxn^{-1} 10 mM PEFABLOC SC solution were dissolved in an appropriate volume of the autoclave sterilized partial ATASE extraction buffer, given that 500 µL is required for each sample. The 10 mM PEFABLOC SC solution was prepared by dissolving 9.6 mg PEFABLOC SC (Sigma) in 4 mL H_2O; PEFABLOC SC, which is an irreversible inhibitor of serine proteases, should remain stable in solution for up to 6 months at 4°C. The presence of EDTA or GSH in the ATASE activity extraction buffer allows storage of the extracts at -80°C for one month without significant deterioration. In the absence of either EDTA or GSH, up to 50% of a sample's ATASE activity can be lost overnight even if stored on ice or in a freezer (Shiio and Ishii, 1969).

The complete ATASE reaction buffer consists of 10 mM $MgCl_2$, 6 mM PRPP, 40 mM NaF and 10 mM L-Gln. 60 mL of partial ATASE reaction buffer was prepared by dissolving 122.0 mg $MgCl_2.6H_2O$ in 60 mL H_2O. To complete the buffer, 0.168 mg rxn^{-1} NaF, 0.146 mg rxn^{-1} L-Gln and 0.300 mg rxn^{-1} PRPP (Sigma) were dissolved in an appropriate volume of the autoclave sterilized partial ATASE extraction buffer, given that 100 µL is required for each sample. NaF functions as an inhibitor of

serine/threonine phosphatases. As 100 µL of protein extract is combined with 100 µL of the complete buffer to perform the glutamine amidotransferase reaction, the final working concentration of each component in the reaction mixture is 5 mM $MgCl_2$, 3 mM PRPP, 20 mM NaF and 5 mM L-Gln.

The partial glutamate dehydrogenase reaction buffer contains 100 mM Tris/HCl, pH 8.0, and 2 mM EDTA. 150 mL of this solution was prepared by dissolving 2364 mg Tris/HCl and 111.7 mg EDTA in 150 mL H_2O, with adjustment to pH 8.0 using 10 M KOH. The remaining components are only combined at the time of the spectrophotometric assay, with each reaction prepared individually. The assay also requires 1 U rxn^{-1} L-GDH (Sigma) and a 25 mM APAD stock solution, prepared by dissolving 0.497 mg rxn^{-1} APAD (Sigma) in an appropriate volume of H_2O. Additionally, positive control reactions may be provided by performing a reaction with a solution of L-Glu added in place of the sample. For this purpose, 1.0 mL of 87.5 mM L-Glu was prepared by dissolving 12.6 mg of L-Glu in 1.0 mL of H_2O; when 175 µL of this solution is used in a GDH reaction, a working concentration of 20 mM L-Glu will be achieved. Due to the poor solubility of the amino acid L-Glu the solution was heated to 80° C for 45 minutes and vortexed vigorously. Negative control reactions were performed by adding 175 µL of H_2O in place of the sample.

For protein extraction, wild-type and *alx13* plants at 30 and 33 days' of age respectively were used; at this age all specimens were at the ~14 leaf stage. All tissue samples consisted of the 2nd through 6th leaf pairs of a single plant. As depicted (Fig. 3*C*, *D*), each leaf was dissected into three sections: the leaf tip and midvein, or medial sector, and the two edges of the leaf blade, or lateral sectors, corresponding to the typical areas of *alx13* leaves that are green and chlorotic respectively. During leaf dissection, medial and lateral leaf fragments were held in microfuge tubes cooled to -20°C in a freezer box held on ice; after collection of all wild-type or *alx13* pooled sectors, the weight of each sample was determined before immediate freezing in liquid N_2. Tissue collection was performed during the latter half of the photoperiod.

To extract protein, 500 µL of complete ATASE extraction buffer and a sterile ⅛" steel ball were added to each frozen sample, followed by homogenization in a Tissue Lyser (Qiagen) at 25 Hz for 90 seconds using Tissue Lyser Adapters (Qiagen) pre-chilled on

ice. The homogenates were centrifuged at 14000 rpm for 30 seconds at 4˚C, and the supernatants transferred to clean microfuge tubes. All solutions and sample extracts were kept on ice at all times.

To assay the glutamine amidotransferase activity of the extracts, 100 µL of sample was added to 100 µL of ATASE reaction buffer, mixed by pipetting. The reaction mixture was incubated at 30˚C for 40 seconds to allow the glutamine amidotransferase reaction to take place. Reaction was terminated by incubation of the samples in a dry heat bath at 80˚C for 3 minutes. The reaction mixtures were then immediately cooled on ice, and then centrifuged at 14000 rpm for 2 minutes at 4˚C.

The amount of PRPP converted to PRA by ATASE was determined through assessment of Glu levels in the ATASE reaction mixtures. Each GDH reaction mixture consists of 375 µL of 100 mM Tris/HCl 2 mM EDTA partial buffer, pH 8.0, 149 µL of H_2O, 21 µL of 47 U μL^{-1} L-GDH, 30 µL of 25 mM APAD, and 175 µL of ATASE reaction mixture. This results in working concentrations of 50 mM Tris/HCl, 1 mM EDTA, 1 U L-GDH and 1 mM APAD in each reaction mixture. In negative and positive control reactions, the ATASE reaction mixture is replaced by 175 µL of H_2O or of 87.5 mM L- Glu respectively. As the amount of Glu in each ATASE reaction mixture is measured as the rate of conversion of $APAD^+$ to APADH, as represented by an increase in A_{363}, all components were maintained separately on ice until immediately before each sample was to be assessed.

Spectrophotometric measurements were performed using a Cary 1 spectrophotometer (Varian) and the Cary WinUV Kinetics application (Varian, version 3.00). In the software's Setup menu, the target Wavelength was set to 363 nm. The Spectral Bandwidth and Signal Averaging Time settings were set to 1.0 nm and 0.100" respectively. In the Collect Timing box, a Cycle Time of 0.05 and a Stop Time of 5' were selected; this corresponds to 20 measurements min^{-1} over a 5' period. The spectrophotometer was zeroed to a solution of 75 µL of 100 mM Tris/HCl 2 mM EDTA partial buffer, 149 µL of H_2O, 21 µL of 47 U μL^{-1} L-GDH and 30 µL of 25 mM APAD (*i.e.* a GDH reaction mixture prior to the addition of a sample).

An appropriate number of partial GDH reaction mixtures were prepared by combining 375 µL of 100 mM Tris/HCl 2 mM EDTA partial buffer and 149 µL of H_2O in 1.5 mL microfuge tubes. 5' prior to performing measurement on a sample, 175 µL of the sample ATASE reaction mixture was added to the partial GDH reaction mixture. Immediately before measurement, 21 µL of 47 U μL^{-1} L-GDH and 30 µL of 25 mM APAD were also added to the reaction mixture. The entire reaction mixture was then transferred to a 1.0 mL quartz cuvette and placed in the spectrophotometer. After selecting the Read option, the reaction mixture was allowed to stabilize for 10" before selecting the Start option to begin measurements; this delay avoids small fluctuations in the A_{363} reading that may result from the movement of the sample. ATASE activity was expressed as the maximum linear rate of increase in A_{363}; refer to Supplementary Fig.S5 for an example of data obtained from a glutamine dehydrogenase activity assay. In between measurements, the cuvette was rinsed 4 times with H_2O.

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Table S1. Real-time RT-PCR primers and Universal ProbeLibrary probes used for quantitative transcript analysis

Target transcript	Universal Probe Library probe	Primer sequences
CYP5	UPL 103	5'-GGCAGTTCCTAAAACTGCAGAA-3' 5'-TTCCCTTGTAGTGTAGAGGTTTCC-3'
APX2	UPL 49	5'-CATTTCATCCTGGTAGACTGGAC-3' 5'-CACATCTCTTAGATGATCCACACC-3'
ATASE1	UPL 60	5'-TTCTTCTTACACCAATCAACCTCTC-3' 5'-ATGGGGAGGTTTAAGGAAGG-3'
ATASE2	UPL 35	5'-TCTCTCCTCTTCCGGCTTCTA-3' 5'-AAACTCTGAGCGGAAACGAA-3'
ATASE3	UPL 48	5'-GACCAGTCGAGTCACACAGG-3' 5'-CAGACTCTGTTTTGGTCACAGG-3'
PP2A	UPL 120	5'-GACCGGAGCCAACTAGGAC-3' 5'-AAATGCGACAAAACTTGGTAACT-3'
APX1	UPL 49	5'-TCCCTACCATCTCTTTTGCTG-3' 5'-GTTGGGGCTTGTCCTCTCTT-3'
CAT2	UPL 25	5'-TCTGGTGCTCCTGTATGGAA-3' 5'-TGGTAATCCTCAAGAAGGATAGGA-3'
ZAT12	UPL 103	5'-CCCACGGTGACTACGTTGA-3' 5'-TCAAATTGTCCACCATCCCTA-3'
LHCB1.2	UPL 110	5'-CCCATTGGGTCTTGCTACC-3' 5'-CCGTTCTTGAGCTCCTTCAC-3'
RBCS	UPL 8	5'-CGCTCCTTTCAACGGACTTA-3' 5'-AGTAATGTCGTTGTTAGCCTTGC-3'
RD29A	UPL 69	5’-ACGTCGAGACCCCGATAAC-3’ 5’-CAATCTCCGGTACTCCTCCA-3’