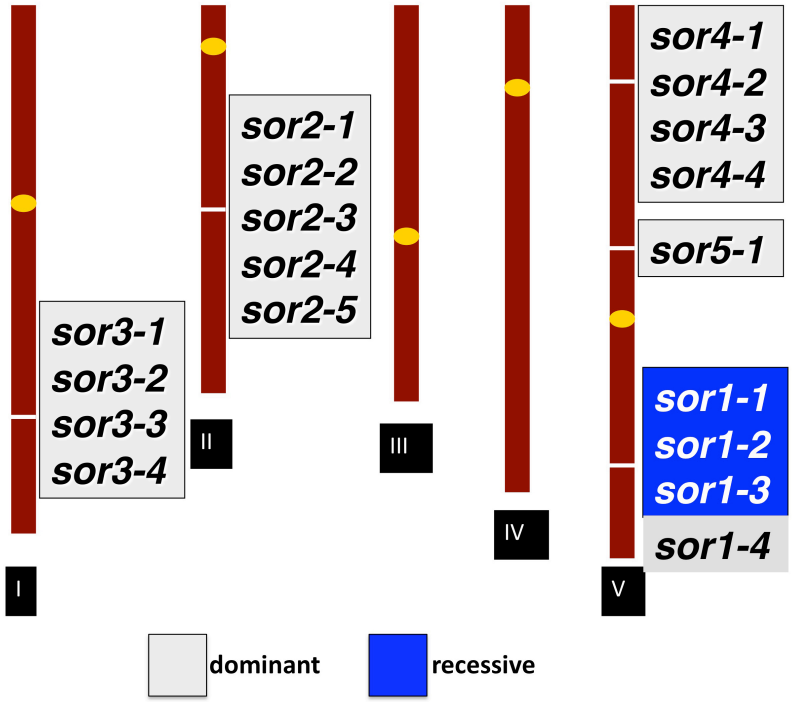
***root uv-b sensitive* Mutants are Suppressed by Specific Mutations in ASPARTATE AMINOTRANSFERASE2 and by Exogenous Vitamin B6**

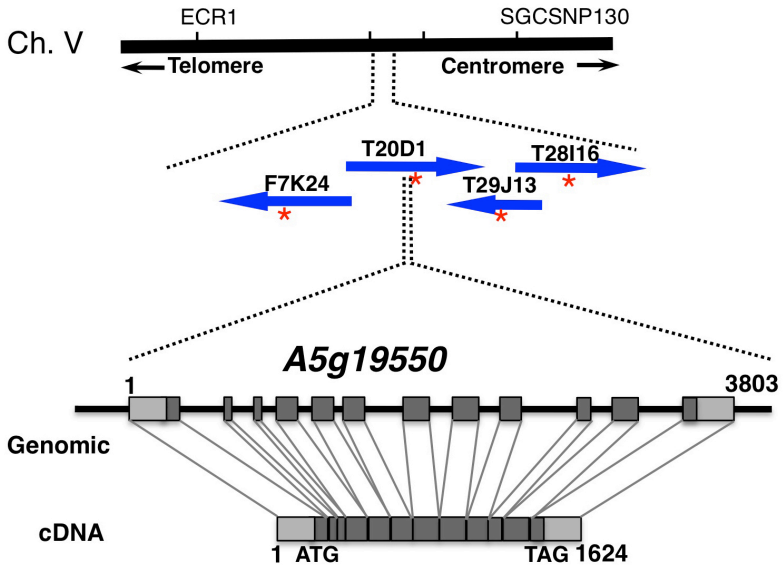
By Leasure et al.,

Supplement Figures



Supplemental Figure 1. A total of 75 individual *sor* mutants have been identified. Seeds of homozygous *rus1-1* (or *rus1-2*, *rus2-1*) were treated with EMS and their M2 seedlings were screened for individuals that show elongated troot growth under the light. Allelic analysis grouped these *sor* mutants into five specific loci on three chromosomes. Centromere positions are indicated by yellow circles and chromosome numbers are marked as I, II, III, IV, V. Multiple alleles have been identified for all loci except *sor5*. Three alleles of *sor1* show recessive suppression (blue) and the rest mutations show dominant suppression (grey). *sor1-1, sor1-2, sor1-3,* and *sor1-4,* are corresponding to *asp2-11, asp2-12, asp2-13,* and *asp2-14,* respectively.

A



B **List of selected markers used for mapping:**

Rough Mapping
CIW8 SSLP) marker:
primer 1: TAGTGAAACCTTTCTCAGAT
primer 2: TTATGTTTTCTTCAATCAGTT

Fine Mapping

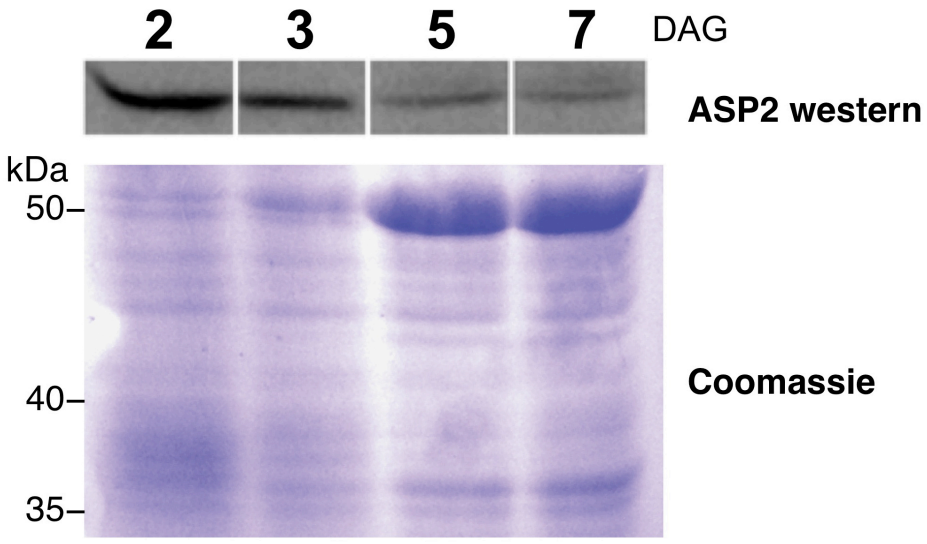
F7K24 (1.6%) CAPS marker:
F7K24-Msp1-F GAAAAATGAGTGAGAGCAGCGG
F7K24-Msp1-R TGATTCAAGACCAGGAGCAGCC

T29J13 (1.7%) SSLP marker:
T29J13sslp-F TCTCACCGACTCAAGTATCCA
T29J13sslp-R ATCGCTTTAGTGATTGTAACTGTC

T20D1 (0.2%) CAPS marker:
T20D1-EcoRV-F CGGAAGTCGCAGAAAACAAACC
T20D1-EcoRV-R CGTTGGAACTGAAAAGGTGGG

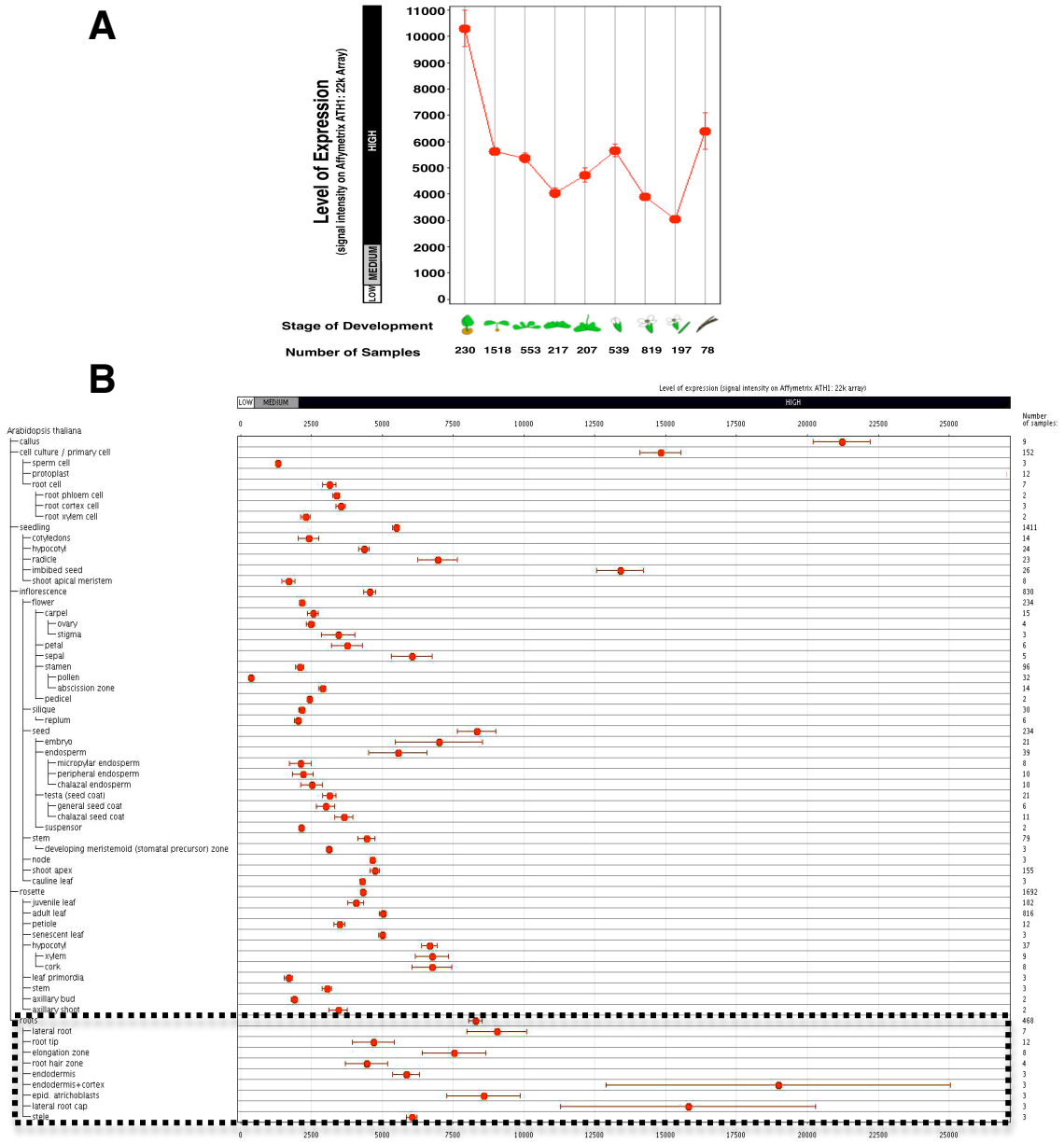
F28I16 (3.7%) CAPS marker:
F28I16-Dra1-F ATTTGCCCGACTGCTCCTTCTC
F28I16-Dra1-R CTTACTGTCAGCAGGCTCATTCG

Supplemental Figure 2. Map-based cloning of *sor1-2* (*asp2-12*). (**A**) The *sor1-2* mutation mapped to a 100-kb region on T20D1 on chromosome 5. Values = number of recombinant chromosomes/total number of chromosomes tested. Markers in the adjacent BACs units are indicated by *. The gene structure of ASP2 (*At5g18550*) is shown on the bottom. Shaded boxes = exons. Position 1 = transcriptional initiation site. 1624 and 3803 = positions relative to the transcriptional initiation site in genomic and cDNA, respectively. The ATG start codon and stop codon (TAG) position are indicated. (**B**) List of markers used for rough and fine mapping. The percent values are number of recombinant chromosomes/total number of chromosomes tested.

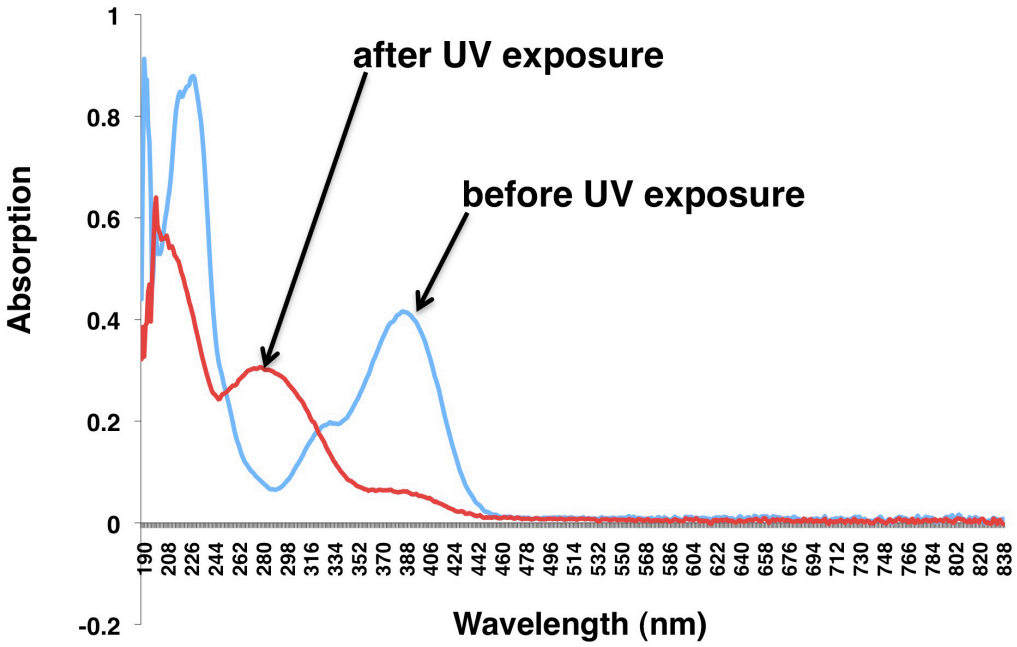


(DAG: Day after germination)

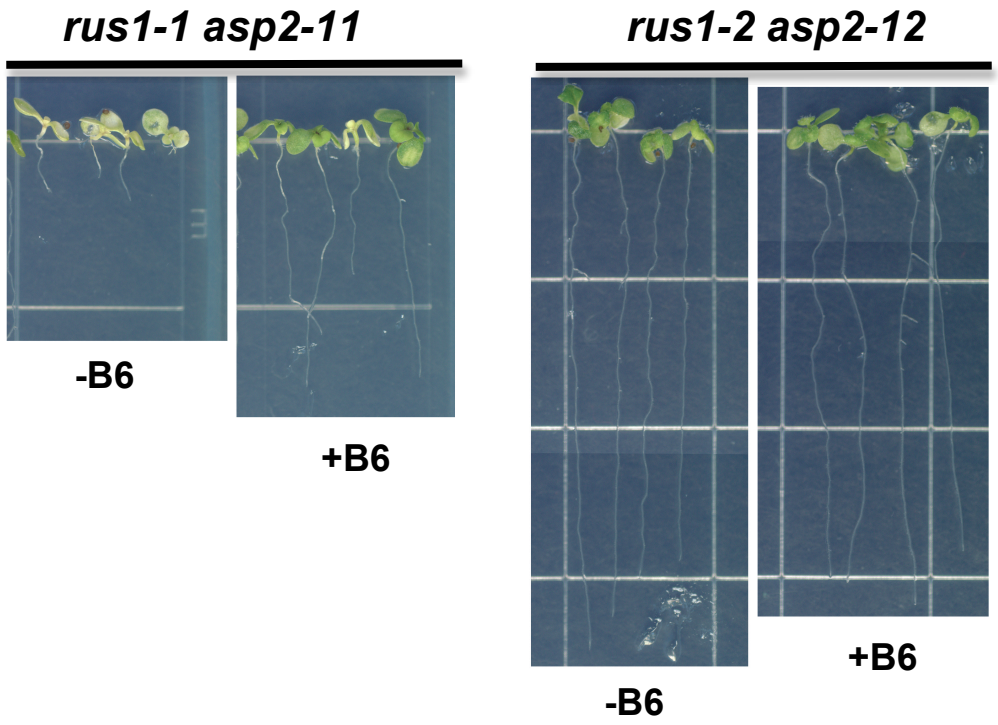
Supplemental Figure 3. ASP2 expression during early seedling development. Top = Western blot of wild-type protein extracts of two, three, five, and seven day old whole seedling protein extracts. Bottom = A section centered around ASP2 of the same protein extracts stained with Coomassie. Storage proteins (2-day-old) between 35-40 kDa and Rubisco protein (5- and 7-day-old) at 50 kDa are prominent.



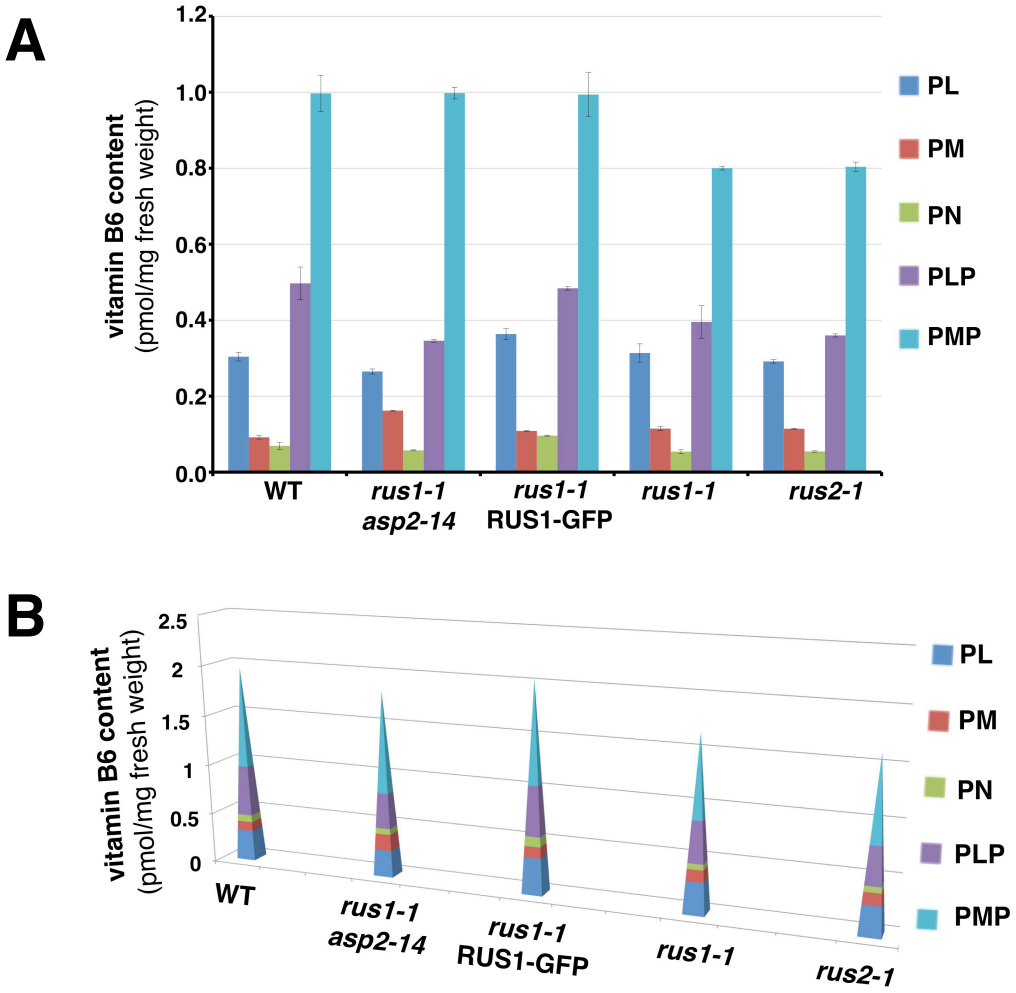
Supplement Figure 4. Scatterplots of microarray analysis showing *ASP2* expression profiles during Arabidopsis development and in different Arabidopsis tissues. Number of samples used to calculate *ASP2* expressions are indicated. Average expression values were calculated from all arrays in the focused array selection (Affymetrix ATH1: 22k array). Analyses were carried out at the Genevestigator online site (www.genevestigator.com) using publicly available data (Hruz et al., 2008). **A.** *ASP2* expression levels during Arabidopsis development. The standard nine stages of development are indicated by cartoons below. B. *ASP2* expression levels in various Arabidopsis tissues. Left column lists the ontology tree structures of various Arabidopsis tissues and cell cultures. *ASP2* expression in roots is well within the range of high expression signal intensity (boxed by dashed line).



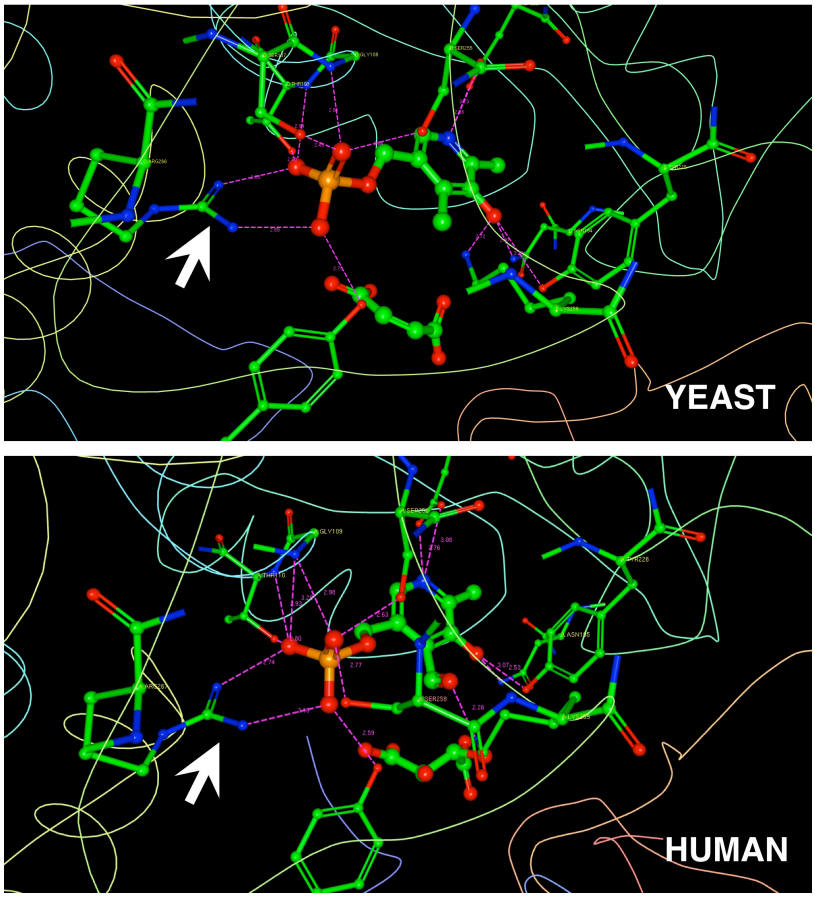
Supplemental Figure 5. Ultraviolet light exposure irreversibly convert pyridoxal 5'-phosphate (PLP) to a photoproduct. The absorption spectrum of 10 mM PLP in PBS (PH 7.0) is shown in blue (before UV exposure). The same solution was exposed to UV-B for 10 minutes and its absorption spectrum was measured (in red). A Chromata-VUE Transilluminator equipped with UVB tube FL15E UV-320 was used as the UV-B source (Model TM-36, UVP) (Tong et al., 2008, PNAS 105: 21039-21044). The absorption spectrum of the UV-B exposed solution is irreversibly altered (after UV exposure).



Supplemental Figure 6. Responses of different *asp2* suppressors to vitamin B6. Seeds of *rus1 asp2-11* (*asp2-11*) or *rus1 asp2-12* (*asp2-12*) double mutants were placed on MS media that were supplemented either with 10 μg/ml vitamin B6 (+B6) or without B6 (-B6). Plates were vertically placed in a growth chamber with standard light cycles (16-hr light, 8-hr dark). Images of 10-day-old seedlings are shown here.



Supplemental Figure 7. HPLC measurements of B6 vitamers in WT and various mutans. B6 viatmers were extracted from 14-day-old seedlings of WT (WT), *rus1-2 asp2-14* (*rus1-2 asp2-14*), *rus1-1* RUS1-GFP complementation line (*rus1-1*RUS1-GFP), *rus1-1*(*rus1-1*) and *rus2-1* (*rus2-1)*. **A.** Levels of individual vitamers in the five indicated genotypes. (Error bar = SE). **B.** Total vitB6 levels in the five indicated genotypes. Seedlings were grown for 14 days in a growth chamber with standard light cycles (16-hr light and 8-hr dark) (light) on MS media that were supplemented with the standard 1X MS vitamin (final concentrations: glycine, 2 mg/L; myo-inositol, 100 mg/L; nicotinic acid, 0.50 mg/L; pyridoxine HCl, 0.50 mg/L; and thiamine HCl, 0.10 mg/L). Both un-phosphorylated (pyrdoxal – PL; pyridoxamine – PM; pyridoxine – PN) and phosphorylated (pyridoxal-5'-phosphate – PLP; pyridoxamine 5'-phosphate – PMP) B6 vitamers were determined from standard curves that were constructed by known standards under the same experimental condition. Levels of PNP (pyridoxine 5'-phosphate) were not detectable in the analyzed tissues. All assays were carried out according to established procedures (see Material and Methods).



Supplemental Figure 8. The dominant suppressor, asp2-14 (R259H), carries a substitution in a conserved Arginine residue essential to bind the vitamin B6 cofactor. This 100% conserved Arginine residue is critically important in forming the phosphate binding cup via two hydrogen bonds (arrows). Top panel, yeast ASP; lower panel, human GOT1. Images are generated using Ligand Explorer (http://www.kukool.com).