**SHORT LEGENDS FOR SUPPORTING INFORMATION**

Supplemental Figure 1. pH and Mg2+ optima for IMPL1 and IMPL2.

Supplemental Figure 2. Gene Expression of IMP, IMPL1 and IMPL2.

Supplemental Figure 3. Western Blot Analysis of IMP:GFP, IMPL1:GFP and IMPL2:GFP Fusion Proteins.

Supplemental Figure 4. Subcellular Location of IMPL1, and IMPL2 N-terminal Signal Peptide Fused to GFP Protein.

Supplemental Figure 5. Hydrolysis of L-Histidinol 1-P.

Supplemental Figure 6. GC-MS of Metabolites.

**Supplemental Figure 1. Mg2+ and pH dependence of purified IMPL1 and IMPL2.** **(A)** Purified IMPL1 and IMPL2 enzymes were incubated in Tris-Cl (pH 7.5) with 0.5 mM D-Ins 1-P and 0.5 mM Histidinol 1-P, respectively, for 10 min with varying concentrations of MgCl2. Enzyme activity was determined by phosphate release assay. **(B)** As in A, except that pH was varied with Tris-Cl buffers and 4 mm MgCl2 was present in all assays.

**Supplemental Figure 2. Gene Expression of IMP, IMPL1 and IMPL2.**

Genevestigator (https://www.genevestigator.com/gv) microarray data was queried to determine IMP, IMPL1 and IMPL2 expression in various tissues. Data from Genevestigator database was analyzed and plotted. The different tissues are indicated on the x-axis. Error bars indicate standard deviation.

**Supplemental Figure 3. Western Blot Analysis of IMP:GFP, IMPL1:GFP and IMPL2:GFP Fusion Proteins.** Denaturing SDS-PAGE and protein gel blot analysis of plant extracts using anti-GFP antibody. 23-d-old rosettes were taken from transgenic plants expressing IMP:GFP or IMPL1:GFP or IMPL2:GFP under the control of 35S promoter. Ponceau S staining of the blot is shown in the bottom panel and the is due to staining of Rubisco at the expected molecular mass of 55 kD.

**Supplemental Figure 4. Subcellular Location of IMPL1, and IMPL2 N-terminal Signal Peptide Fused to GFP Protein.** Single optical sections of transgenic plants expressing IMPL2 N-ter:GFP **(A)**, IMPL1 N-ter:GFP **(B)**,**(C)**. All images were taken of root hairs and guard cells with differential interference contrast (DIC). Bars = 20 μm.

**Supplemental Figure 5. Hydrolysis of L-histidinol-P in Metabolite Extraction Procedure**. Leaves of 23-d-old wild-type plants were harvested and during the extraction procedure 100 μmoles of histidinol 1-P was added as described in Methods to assess the degree of hydrolysis to histidinol. Standard error is indicated (biological n=3). \*\* p value < 0.001.

**Supplemental Figure 6.** Whole 7-d-old seedlings were extracted and GC-MS was used to quantify the indicated metabolites as described in Methods. Means and SE are presented. Data from three independent biological replicates were averaged.

**SUPPLEMENTAL METHODS**

### Seedling Root Growth and Seed Germination Assays

Seeds were sterilized with 30% Clorox, rinsed, and plated on 0.8% agar plates containing 0.5xMS medium (pH 5.8) and 1% sucrose. As indicated, plates contained histidine, histidinol, inositol, glutamine, NiCl, galactose and glucose (all from Sigma-Aldrich). Seeds were stratified on plates at 4°C for 3-4 days before germination. A seed was considered as germinated when the radical protruded from the seed coat. Root length was measured on vertical plates. Three plates each of 40 seeds per line were scored in germination assays. Two to three plates with 30 seeds per line were scored for root growth. For germination assays, seeds were plated on medium containing 0 mM or 0.04 mM histidine and germination was scored over 72 hrs. For root length measurements seeds were plated on 0 mM, 0.01 mM, 0.02 mM, 0.04 mM, 0.4 mM and 0.8 mM histidine and 0.04 mM Glutamine and 30 mM inositol for controls.

### Genomic PCR Analysis of *impl2* Mutants

Seeds for *impl2-3* (SAIL\_146\_E09), *impl2-4* (SAIL\_35\_A08), and the corresponding wild-type plant, CS60000, were obtained from the ABRC at Ohio State University. Genomic DNA from segregating plants was screened by PCR using the primers noted below and then sequenced to verify T-DNA insertion sites. Genomic DNA was isolated from leaves of soil-grown plants using a DNAeasy kit (QIAGEN Inc., Valencia, CA). DNA from segregating plants was screened utilizing the SAIL left border (LB) primer and IMPL2 gene specific primers using annealing temperatures of 53-57°C for amplification. For *impl2-3* (SAIL\_146\_E09), the T-DNA insertions were verified by sequencing both ends of the T-DNA, and were found to consist of two tandem T-DNAs, right border adjoining right border. The insertion site is in Exon 1 after nucleotide #24 from translation start site. Sequencing of the other T-DNA end indicated that several base pairs have been deleted. For *impl2-4*, (SAIL\_35\_A08), the two tandem T-DNAs, right border adjoining right border, are inserted in Exon 1 at nucleotide #66 from the translation start site.

**Constructs and Imaging**

For IMP:GFP, IMPL1:GFP and IMPL2:GFP, seedlings from two independent homozygous lines were identified on kanamycin screening and GFP production was observed using a Zeiss Axio imager microscope equipped with fluorescence optics. Also, two independent homozygous complemented lines with detectable GFP expression in the *impl2-3* mutant background with IMPL2:GFP were identified and used in metabolite analyses. Co-localization experiments were performed by stably transforming the IMPL2:GFP homozygous lines with a plastid-mcherry marker that is fused to the signal peptide of Rubisco large subunit (Nelson et al., 2007). For screening, three day-old seedlings were used for imaging utilizing Axiovision software (Zeiss). Photographs were taken with a Zeiss MC100 camera by using an excitation filter set of 540 to 580 nm for GFP, consisting of a dichroic mirror of 595 nm and a barrier filter of 600 to 660 nm. The putative signal peptide of IMPL1 (231 bp) and IMPL2 (228 bp) were fused to the N-terminus of eGFP using the previous Gateway cloning method and images were taken as described previously.

**GUS constructs and Imaging**

Intergenic regions containing promoters for IMP (2000 bp), IMPL1 (2015 bp), IMPL2 (1628bp, 1085bp or 461bp) were amplified from CS60000 genomic DNA by high-fidelity polymerase (Velocity enzyme, BioRad Laboratories, Hercules, CA) with gene-specific primers and cloned into the pENTR/D-TOPO vector (Invitrogen, CA). The resulting clones were recombined into the binary vector pBGWFS7 (Invitrogen, CA) containing a *Egfp:uidA* gene fusion. The gateway recombination was catalyzed by addition of LR clonase according to the manufacture’s instructions (Invitrogen, CA). Transgenic plants were generated as described previously. GUS staining of 1- to 19-d-old plants grown on 0.5x MS agar plates with 1% sucrose or of plant tissues from soil-grown plants was as described (Donahue et al. or Styer et al.), and images were taken using Olympus SZX16 microscope with an attached Olympus DP71 camera with DP Controller software (Olympus Corp., Japan).

For the GUS staining procedure, either seedlings or plant tissues from different developmental stages were placed in GUS staining buffer (0.1% Triton X-100, 50 mM phosphate buffer, 0.5 mM K4Fe(CN)6 x 3 H2O), 0.5 mM K3Fe(CN)6, 2 mM 5-bromo-4-chloro-3-indolyl β–D-glucuronide cyclohexamine) and vacuum-infiltrated for 20 min, incubated in the solution overnight at 37°C, and then washed off chlorophyll with 70% ethanol followed by 95% ethanol extractions at 4°C, according to the protocol previously developed (Jefferson, 1987).

### Confocal Imaging

As described in (Donahue et al., 2010), GFP fluorescence was detected with a Zeiss LSM510 laser scanning microscope (Carl Zeiss) using excitation with a 488-nm argon laser and a 505- to 550-nm band-pass emission filter. Chlorophyll auto-fluorescence was imaged using excitation with a 543-nm HeNe laser and 560-nm band-pass emission filter. Slides were examined with a 340 C-Apochromat water immersion objective lens.

### Protein Blot Analyses

Plant tissues were frozen in N2 (l) and ground into a fine powder using a mortar and pestle. Samples were homogenized in an extraction buffer (50 mM Tris-Cl, pH 7.5, 150 mM NaCl, 5 mM MgCl2, 0.05% Triton X-100, 10% glycerol, 1 mM Dithiothreitol (DTT), and Protease Inhibitor Cocktail for plant extracts, (Sigma-Aldrich)) and centrifuged at 4°C for 2 min at 13.2 k rpm on a table-top microcentrifuge. The supernatant was kept and quantified by BCA assay (Pierce), and mixed with Laemmle gel loading buffer (100 mM Tris-Cl, pH 6.8, 4% Sodium dodecyl sulfate (SDS), 0.2% bromophenol blue, and 20% glycerol), and boiled for 5 minutes. Proteins were separated on 10% or 12% SDS-PAGE gels and transferred to nitrocellulose using a semi-dry transfer apparatus (Bio-Rad Laboratories, Hercules, CA). The nitrocellulose membranes were incubated in 5% non-fat dry milk in 1x TBST blocking solution for 4 hrs at room temperature. For detection of GFP, a 1:5000 dilution of the rabbit anti-GFP (Invitrogen, CA) was used. All membranes were probed with a secondary antibody at 1:2500 dilution of goat anti-rabbit horseradish peroxidase-conjugated antibody (Bio-Rad Laboratories, Hercules, CA). All antibody solutions were in 2.5% non-fat dry milk in 1x TBST. Primary antibodies were incubated overnight at 4°C, while secondary antibodies were incubated for 1 hr at room temperature. The nitrocellulose membranes were washed three times for 20 minutes with 1x TBST (50 mM Tris-Cl, pH 7.5, 0.9% [w,v] NaCl, and 0.01 % [v,v] Tween-20) buffer before and after applying secondary antibody. Membranes were illuminated with the Amersham ECL Plus Western Blotting Detection kit (GE Healthcare, UK) and exposed to X-ray film for detecting signal. To ensure equal loading of proteins, Ponceau S staining of the membranes was used.

### Gas Chromatography Analysis

Seedlings were grown on filter paper soaked with 0.5x MS, pH 5.8 and 1% sucrose. Extractions and semi-quantitative GC-MS analyses of amino acids and sugar metabolites were performed on three biological replicates as described previously (Goyer et al., 2005; Collakova et al., 2008). Briefly, seedlings and tissues were flash frozen in liquid nitrogen and ground into a powder, lyophilized, weighed (5 mg), disrupted with glass beads and extracted with chloroform: 10 mM HCl 1:1 (v/v). Norvaline and ribitol were added to the aqueous phase as internal standards for amino acid and sugar metabolites, respectively. The samples were vortexed and centrifuged at 13.2 k rpm for 5 minutes. A fifth of the aqueous phase was dried under a stream of nitrogen and derivatives were prepared. Amino acids were derivatized in 50 μl of Ν-methyl-Ν-(tert-butyldimethylsilyl)-trifluoroacetamide containing 1% (v/v) tert-butyldimethylchlorosilane (Pierce)/pyridine (1:1 by volume) at 50°C for 1 h. For metabolite profiling, the metabolites were derivatized in methoxyamine-HCl for 2 hrs at 50°C, and N,O-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane; Alltech) for another 30 min at 50°C. One microliter of derivatized samples was injected with pulsed splitless injector (7683B series injector, Agilent Technologies) and separated on an Agilent 6890 series gas chromatograph equipped with a 30-m DB-5 MS+DG column (0.25mm x 0.25 μm) and analyzed in scan mode using an Agilent 5975C (inert XL MSD with triple-axis detector) series quadrupole mass spectrometer (Agilent Technologies). Agilent enhanced analysis software was used for the analysis of data. Helium was used as the carrier gas with pressure-controlled flow set at 10.3 psi, and a linear velocity of 1.19 ml min-1. The injector port was set at 280°C (300°C for amino acid profiling) with 10.3 psi pressure and a rate flow of 24 ml min-1. The oven gradient was set from 75°C to 320°C at 10°C min-1 for metabolite profiling and from 100°C to 300°C at same increments for amino acid profiling. The thermal transfer line to MSD was kept at 250°C. MS was set in a scan mode detecting m/z between 100 to 650 for metabolite profiling and m/z of 50-800 for amino acids.

### Expression of Recombinant Protein

Plasmids containing the genes IMPL1 (At1g31190) and IMPL2 (at4g39120, truncated at the 5’ end, were designated ptIMPL1AE and ptIMPL2AE. The truncation of IMPL1 was accomplished by removing the coding region for the N-terminal 74 amino acids, then replacing the codon for the next amino acid (Gly) with an ATG codon. In a similar fashion, the N-terminal 76 amino acids were removed from IMPL2 by deleting nucleotides and replacing the glutamine codon with an ATG codon. The genes were amplified by PCR from pAtIMPL1H and pAtIMPL2H plasmid templates with primer pairs 5’-ATAggatccATGGCTAAAACCACCGGAAC-3’ (forward)/ 5’-CGCgaattcTTAAAGCTCTGA- TGATAATC-3’ (reverse) and 5’-ATAgattcATGCTTAGCGACACTGAGCTG-3’ (forward)/ 5’-GGCgaattcTCAATGCCACTCAAGTG-3’ (reverse), respectively (lowercase letters indicate restriction sites). The products were digested with *Bam*HI and *Eco*RI and ligated to digested pGEX2T (GE Healthcare). The plasmids are designed to express truncated proteins fused to a C-terminal glutathione S-transferase. The sequences of the plasmids were verified by sequencing.

Overexpression of IMPL1 and IMPL2 was induced in the host strain pREP4 BL21(DE3)\*. A 1.0 L culture with optical density at 600 nm of 0.6, grown in Luria-Bertani medium with 100 µg ml-1 ampicillin and 50 µg ml-1 kanamycin, was induced with 0.1 mM isopropyl-D-thiogalactopyranoside overnight at room temperature without shaking. Cells were harvested by centrifugation and frozen at -80°C. All subsequent steps were performed at 4°C. Cells were resuspended in 20 ml of lysis buffer (50 mM potassium phosphate, 400 mM NaCl, 100 mM KCl, 10% glycerol) pH 7.8, supplemented with 1 mg ml-1 lysozyme, 0.5 mM phenylmethanesulfonyl fluoride, and 1 mM *myo*-inositol. After incubation at 4°C for 35 min, cells were lysed by sonication and Buffer B (1X phosphate-buffered saline, pH 7.3, 1 mM DTT, 0.2% Triton X-100, 56 mM inositol, 5% glycerol) and C (same as Buffer B with 0.1% Triton X-100) were added before centrifugation for 20 min. The clear lysate was incubated for 2 hrs with Pharmacia Glutaminetathione Sephadex (GE Healthcare), washed with 1X phosphate-buffered saline with 0.1% Triton X-100, and then collected in a column. Protein was eluted with 10 mM glutathione in 50 mM Tris-Cl, pH 8.0, and aliquots were frozen at -80°C. Fractions were collected, combined and dialyzed extensively in 50 mM Tri-Cl, pH 7.5, 1 mM MgCl2 and 1 mM DTT. Purified recombinant proteins were frozen in aliquots at -80°C with 10% glycerol. Protein purification and size were estimated by gel fractionation using a 10% SDS-PAGE and pre-stained markers (Bio-Rad).

 **LC/MS/MS Modifications**

A Tosoh Bioscience, LLC, TSKgel Amide-80 HR, 4.6 x 250 mm, 5 micron column was used. The isocratic gradient 65% A : 35% B at 400 μl per minute for 20 minutes was used. A is LC/MS grade water supplemented with 0.1% formic acid. B is LC/MS grade acetonitrile. Dried samples were dissolved in 200 μl of 65% A: 35% B and 5 μl was injected and gradient was created using an Agilent 1100 Series autosampler and HPLC with attached solvent degasser. Mass spectrometer was an ABSciex 3200 Q Trap. Acquisition method was a multiple reaction monitoring (MRM) method in positive ion mode with both quadrupole 1 and quadrupole 3 operating at unit resolution. Curtain, nebulizer and turbo gas were 15, 20 and 40, respectively (arbitrary units). Ion spray voltage was 5500, interface heater was on 40°C and source temperature was 320°C. Source used was a Turbo V electrospray source. CAD (collision activated dissociation) gas was set to high (4.5 x 10-5 torr). Analyst 1.4.2 (ABSciex) was used to collect data, calculate peak areas and generate calibration curves. Areas used for histidine and histidinol were the sums of the areas for both compound specific MRMs.