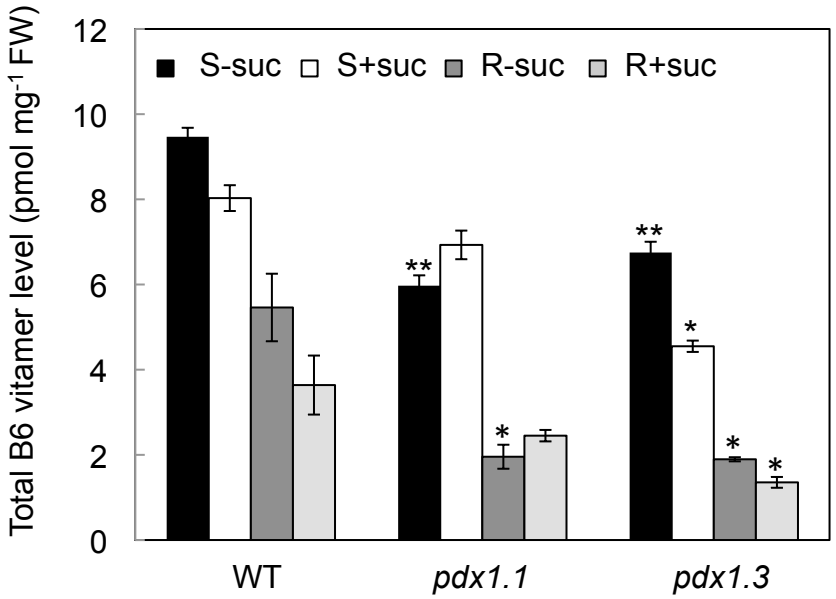
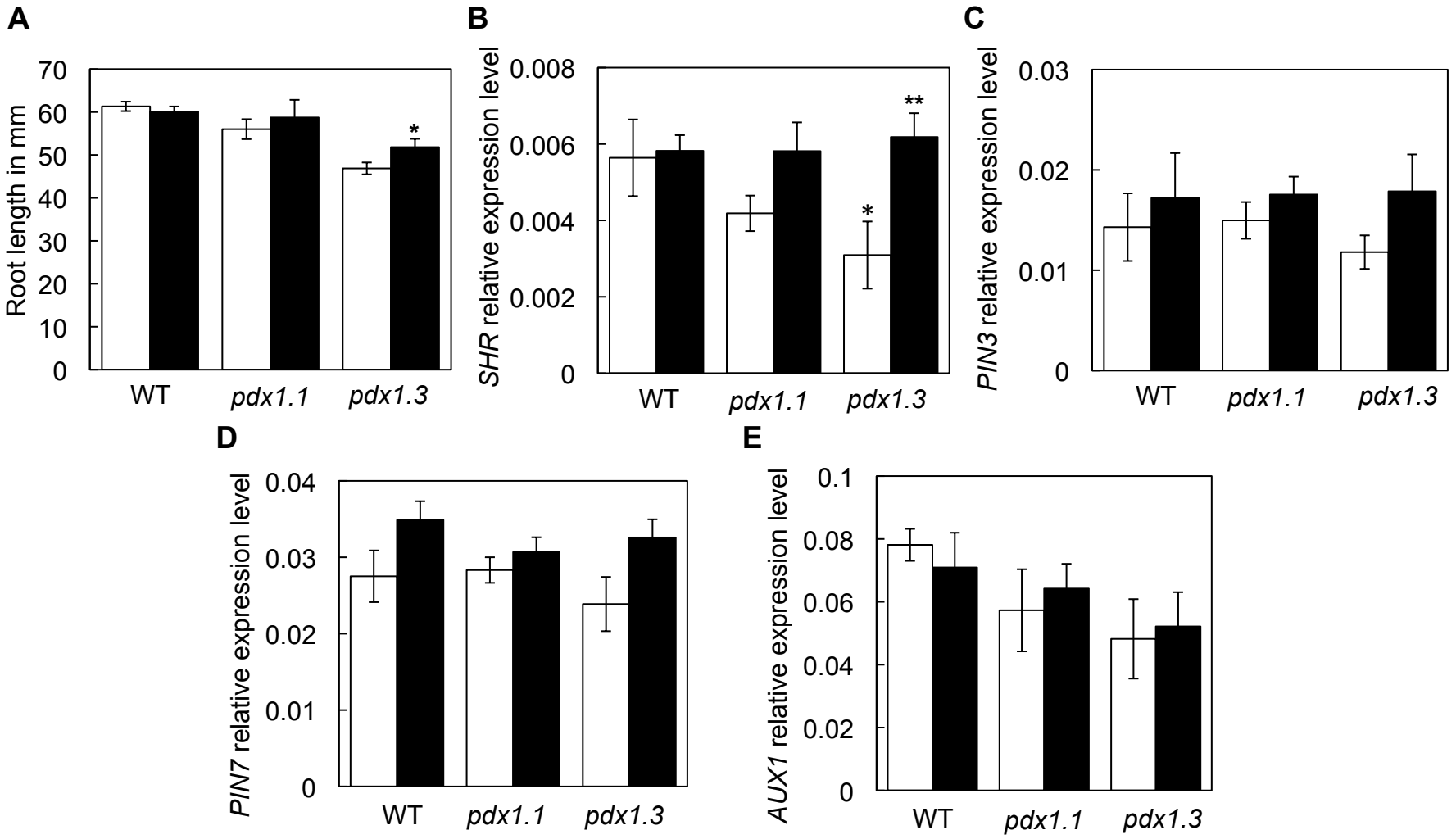
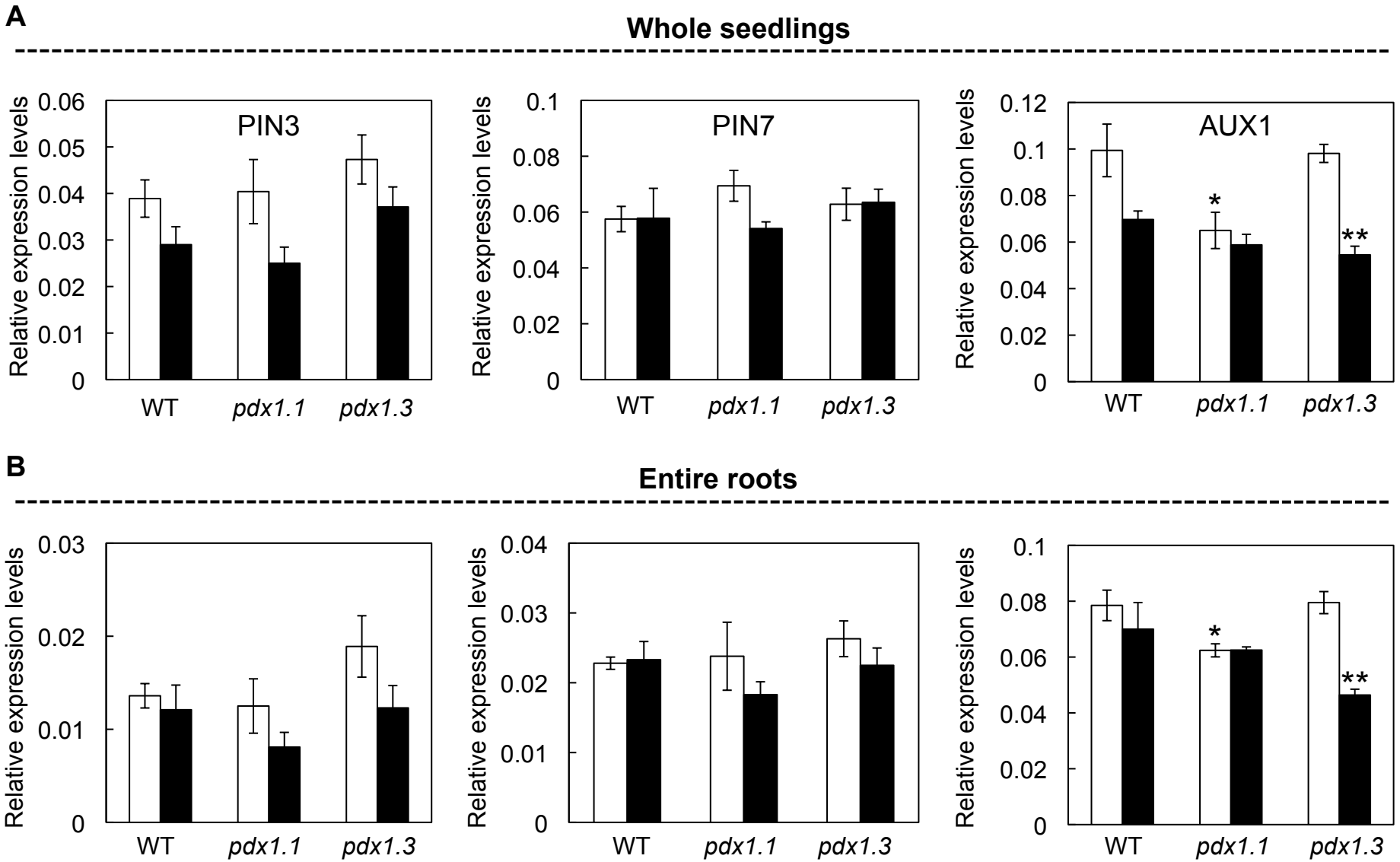
Supplemental Figure 1. Total B_6 vitamer levels in *Arabidopsis* shoots and roots in the presence and absence of sucrose. Wild-type (WT), *pdx1.1* and *pdx1.3* plants were grown for 10 days after germination. Total vitamer levels were determined by HPLC separation and quantification of the different vitamers. The results are the mean values of two independent experiments with at least six technical replicates. Statistically significant differences between the wild-type and the two mutant plant lines within each group of treatment or type of tissue were determined using ANOVA, Dunn's or Bonferroni tests and are indicated by asterisks (* for $P < 0.05$ and ** for $P < 0.001$). S-/+suc and R-/+suc refer to shoots or roots in the absence or presence of sucrose, respectively.



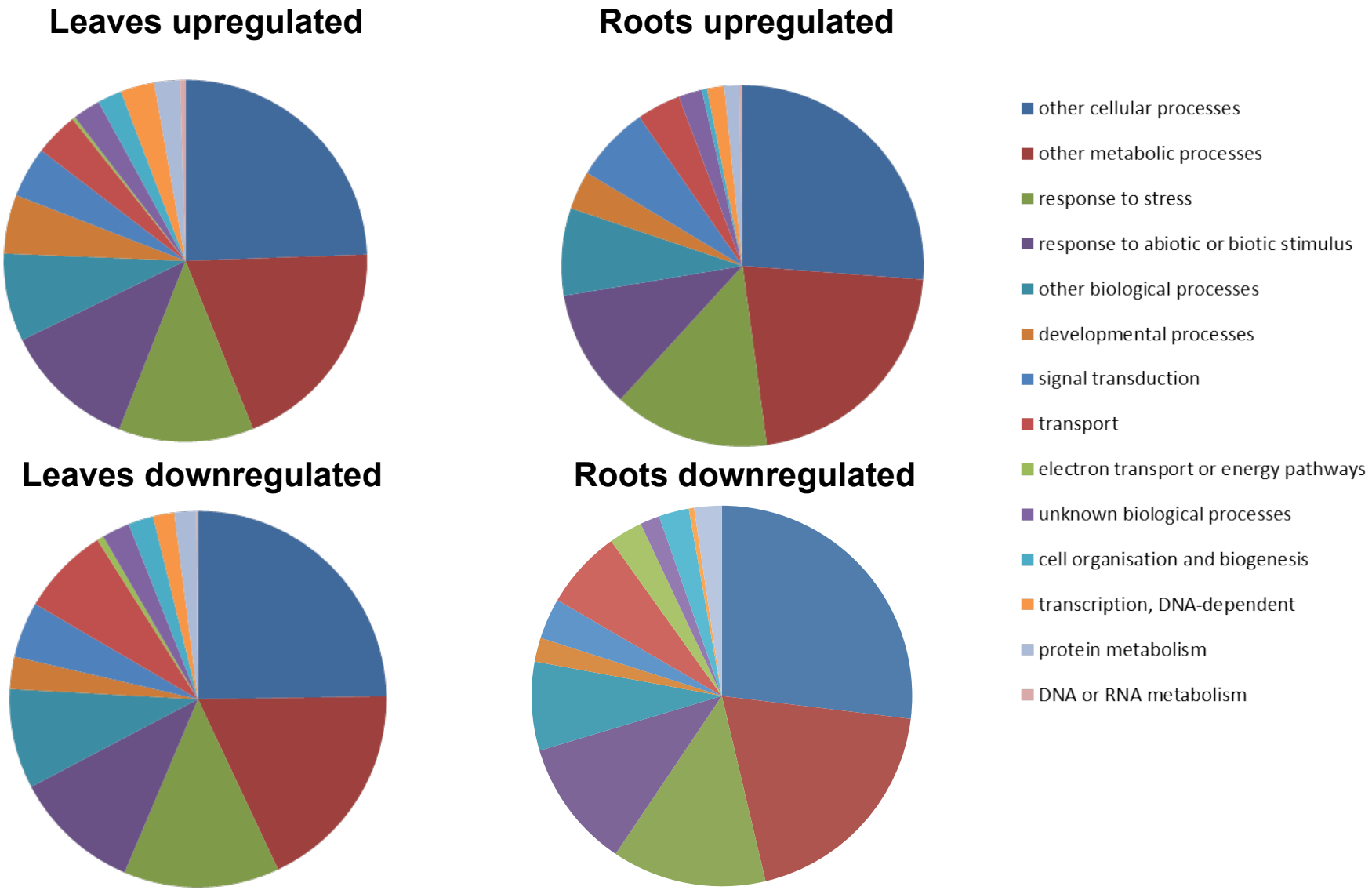
Supplemental Figure 2. Effect of ACC application in the absence of sucrose. (A). Root length of seedlings grown without sucrose either in the absence (white bars) or presence of ACC (5 nM) (black bars). The results are mean values from 2-3 independent biological repetitions each one containing at least 12 replicates per variant. The asterisk indicates statistically significant differences compared to the untreated control for P < 0.001 (ANOVA, Dunn's test). Root length was measured using the ImageJ software (http://imagej.nih.gov/ij/). (B), (C), (D) and (E) Relative expression of *SHR, PIN3, PIN7 and AUX1*, respectively, in the absence (white bars) and presence of ACC (5 nM) (black bars). The results are mean values from 2-3 biological replicates with 3-4 technical replicates each. GAPDH was used as a reference gene. The single asterisk denotes a significant change in *pdx1.3* compared to wild-type (WT). The double asterisk indicates a significant difference compared to the untreated control for P < 0.05 (ANOVA, Tukey test). Seedlings were analyzed 10 days after germination.



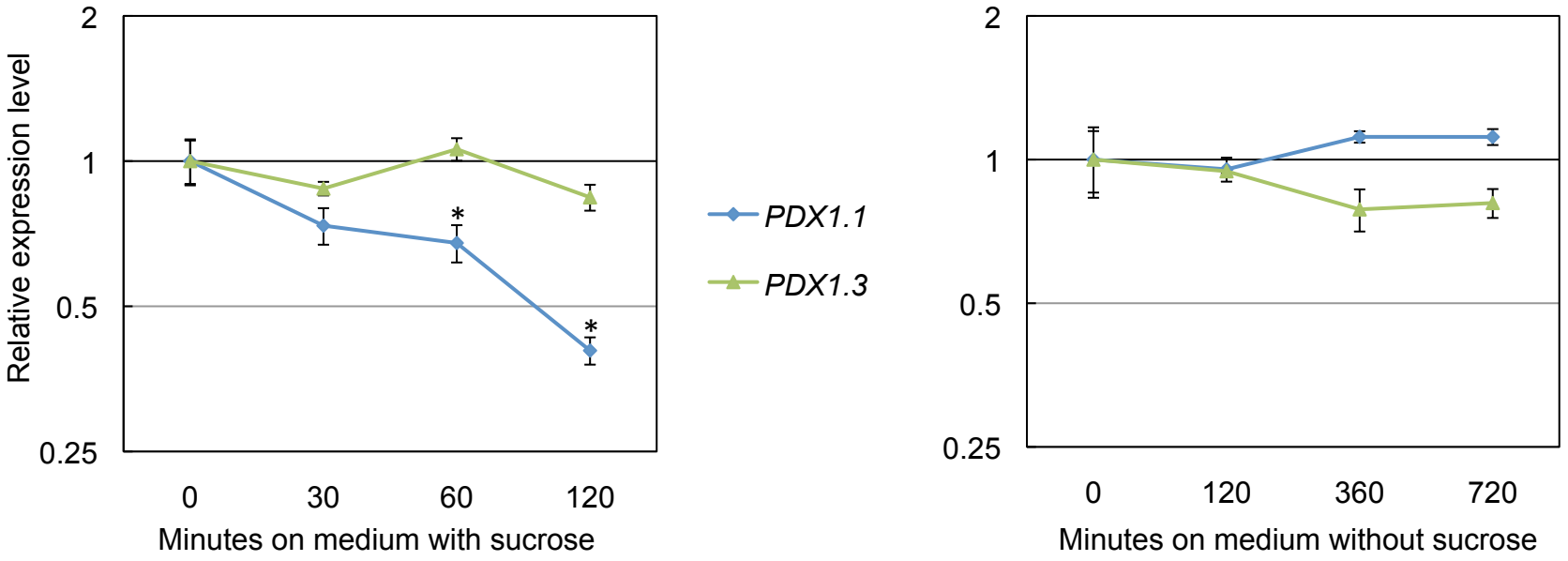
Supplemental Figure 3. Relative expression level of the polar auxin transporters *PIN3*, *PIN7* and *AUX1* in whole seedlings (A) and entire roots (B) 10 days after germination in the absence (white bars) and presence of sucrose (black bars). The results represent mean values of 2-3 biological replicates with three technical replicates each. Error bars show standard error of the mean. Single asterisks denote statistically significant differences between mutant lines and wild-type (WT) under the same conditions $P < 0.01$. Double asterisks indicate statistically significant differences between the treatment groups for $P < 0.001$.



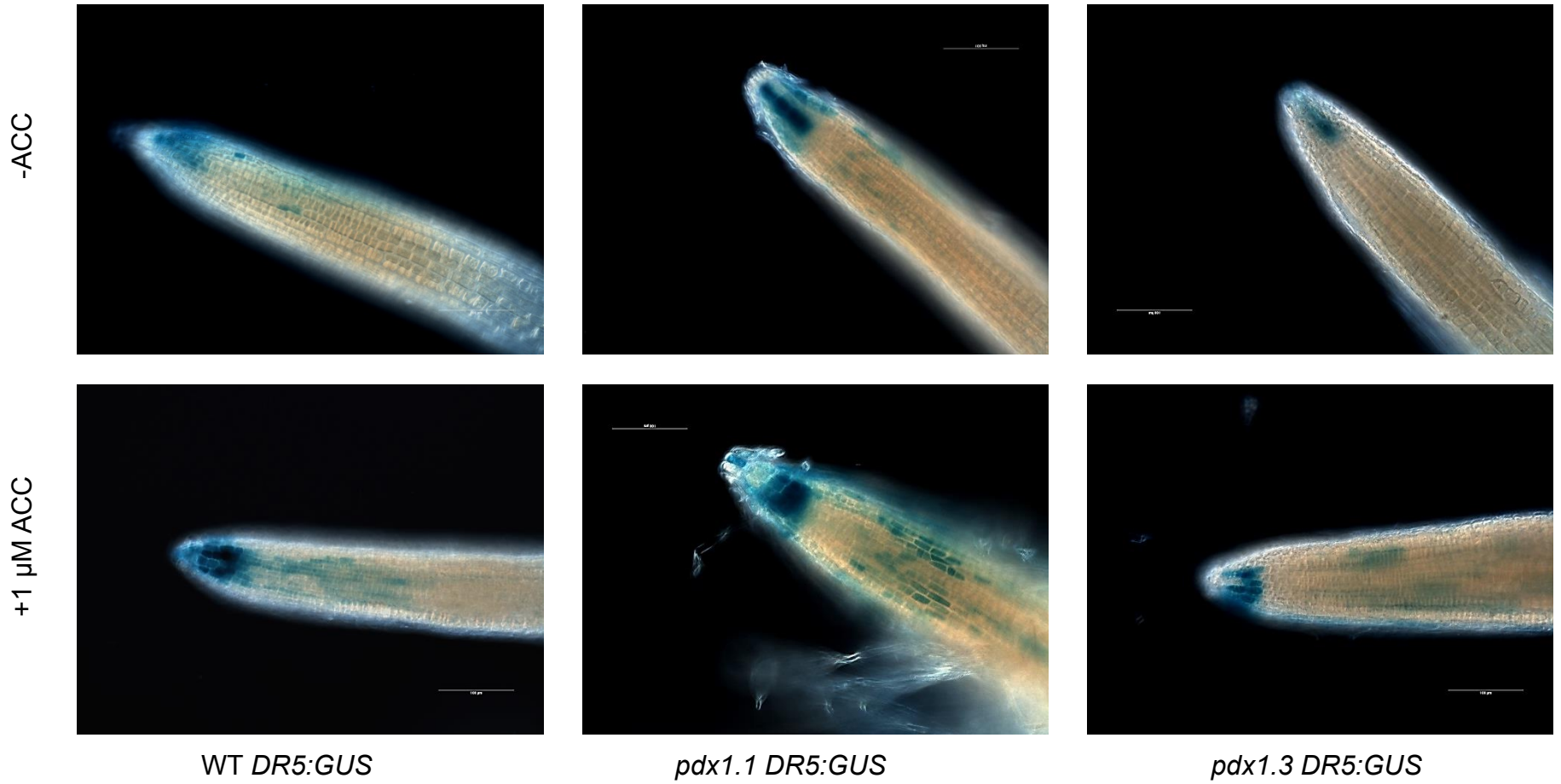
Supplemental Figure 4. Functional categorization of the genes with altered expression in the leaves and roots of the *pdx1.3* mutant *vs*. WT was determined using The Arabidopsis Information Resource http://arabidopsis.org/tools/bulk/go/ (annotation for GO: Biological Process).



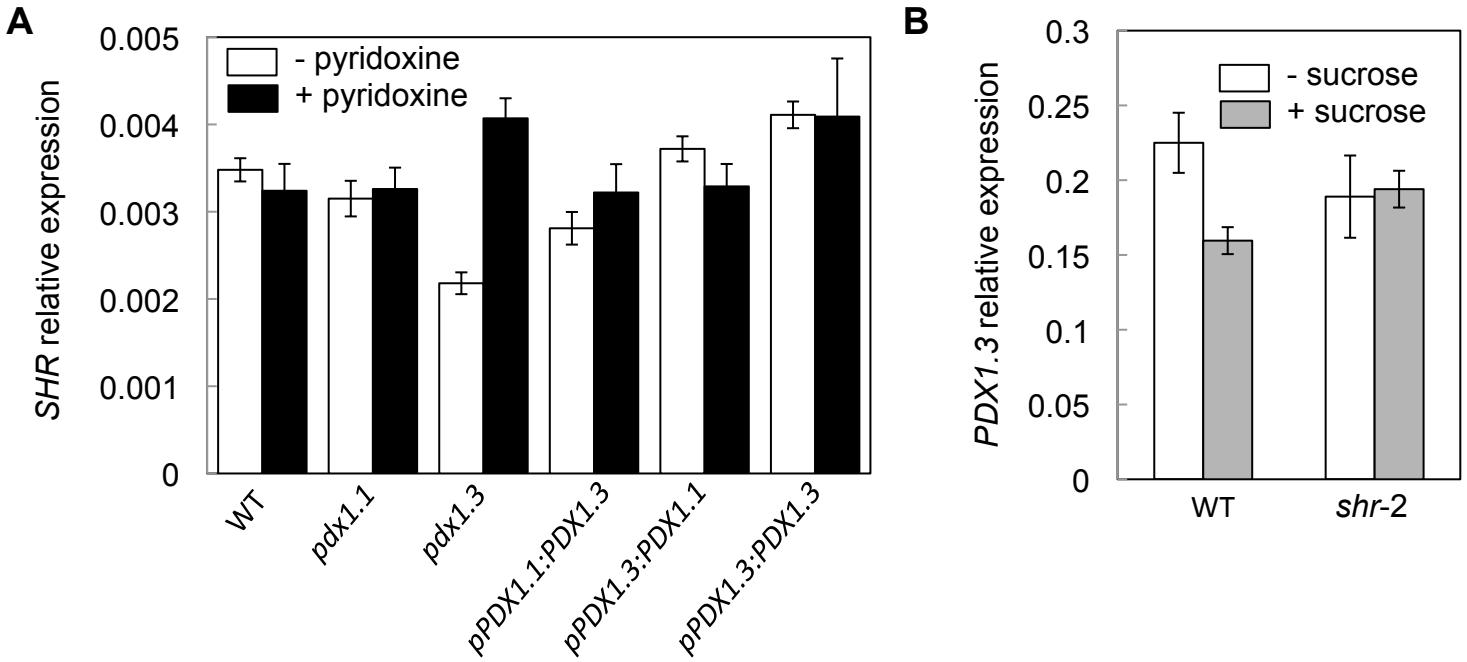
Supplemental Figure 5. Sucrose induced changes in the expression of *PDX1.1* (blue) and *PDX1.3* (green) monitored in wild-type seedlings 4 days after germination, initially grown without sucrose and transferred onto sucrose containing medium for the times indicated. The expression in control plants (transferred to medium without sucrose) are also shown. The results are the mean values from two independent experiments with at least two biological replicates each. Error bars represent the standard error of the mean. Statistically significant differences from time 0 (set to 1) for $P < 0.05$ were determined using the ANOVA, Tukey test and are indicated by a single asterisk.



Supplemental Figure 6. Effect of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) on auxin production and distribution in *Arabidopsis* plants expressing *GUS* under the control of the synthetic auxin inducible promoter DR5. Wild-type (WT), *pdx1.1* and *pdx1.3* plants were grown on medium containing sucrose (1%) and in the presence or absence of ACC. Pictures were captured 5 days after germination. The scale bar represents 100 μm.



Supplemental Figure 7. (A) Relative expression levels of *SHR* in wild-type (WT) lines compared to *pdx1* mutants and the swapped promoter lines in the *pdx1.3* mutant background. Lines carry the promoter region of either *PDX1.1* or *PDX1.3* fused to the *PDX1.1* or *PDX1.3* coding regions (*pPDX1.1:PDX1.3*, *pPDX1.3:PDX1.1* or *pPDX1.3:PDX1.3*, respectively) in the absence or presence of vitamin B_6 supplementation (pyridoxine). (B) Expression of *PDX1.3* in WT compared to the *shr-2* mutant in the presence and absence of sucrose. All the data are mean values from at least two independent experiments and 2 – 3 biological replicates. All the expression analyses were performed using GAPDH as control. Error bars represent standard error of the means.



Supplemental Figure 8. Analysis of *pdx1.3* Arabidopsis lines carrying a mutated version of the putative ethylene response element (mERE) or a mutated version of the putative auxin response element (mAuxRE) in the promoter region of *PDX1.3* compared to the swapped promoter lines carrying the wild-type promoter of *PDX1.1* or *PDX1.3* fused to the *PDX1.1* or *PDX1.3* coding regions (*pPDX1.1:PDX1.3*, *pPDX1.3:PDX1.1* or *pPDX1.3:PDX1.3*, respectively). Five independent lines of *pPDX1.3mERE:PDX1.3* and 4 independent lines of *pPDX1.3mAUxRE:PDX1.3* were analyzed. Root length was measured 10 days after germination and is shown in the top panel. At least 15 seedlings were used per variant and 2 biological replicates. Error bars represent standard errors of the mean. The bottom panel shows the expression level of *PDX1.3* in all variants tested using GAPDH as a control. With the exception of one line 4.3 *pPDX1.3mAuxRE:PDX1.3* (L4.3) and *pPDX1.1:PDX1.3*, all lines showed statistically significant differences for *PDX1.3* expression compared to the mutant line *pdx1.3* ($P < 0.05$) and are the result of 2 technical replicates and 3 biological replicates. Wild-type (WT) and *pdx1.1* are also shown.