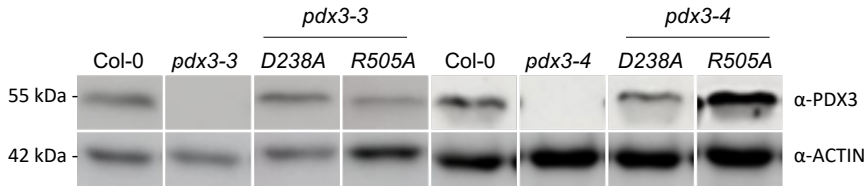


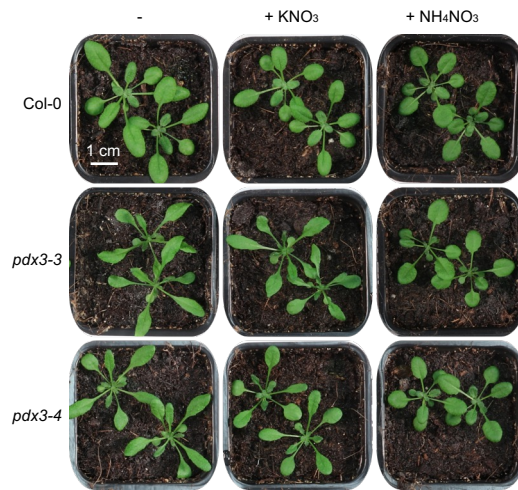
(A)

PDX3 (At5g49970)	MRNVIRRVTTMTFTFLQSPPLPISPSPPQFSLSSSPLSKTQRFITPSQGSRLRTLCTKV	60
PNPO-RP (At2g46580)	-----	0
PDX3 (At5g49970)	IIPNMQDSGSPPLSYLTQREAAEIDETLMGPLGFSIDQLMELAGLSVAASIAEVYKPEEY	120
PNPO-RP (At2g46580)	-----	0
PDX3 (At5g49970)	SRVLAICGPGNNGDGLVAARHLHHFGYKPFICYPKRTAKPLYTGLVTQLDLSLVFVSV	180
PNPO-RP (At2g46580)	-----	0
PDX3 (At5g49970)	EDLPDDLKSKDFDVIVDAMFGFSFHGAPRPPFDDLIRRLVSLQNYEQTLQKHPVIVSV <b>D</b> IP	240
PNPO-RP (At2g46580)	-----	0
PDX3 (At5g49970)	SGWHVEEGDHEDGGIKPDMLVSLTAPKLCAKRFRGPHHFLGGRFVPPSVAEKYKLELPSY	300
PNPO-RP (At2g46580)	-----	0
PDX3 (At5g49970)	PGTSMCVRIGKPPKVDISAMRVNYVSPELLEEQVETDPTVQFRKWFDE---AV--AAGLR	355
PNPO-RP (At2g46580)	-----MGTHVAPWKQLLFGAIEANSHLS	23
PDX3 (At5g49970)	ETNAMALSTANKDKKPSRMVLLKGFDENGFVWF--TNYESKKGSDLSENPAAALFYWE	413
PNPO-RP (At2g46580)	HSSYVQLATIGLNGRPSNRITVFRGFEENS DRIQINTDLRSRKIEELKHCFFSEM CWYFS	83
PDX3 (At5g49970)	ILNROVRIEGPVERIPESESEN-----YFHSRPRGSQIGAI VSKQSSVVPGRHVLY	464
PNPO-RP (At2g46580)	DTWEQFRINGRIEVIDASNP DQTKLQOREKAWFANSLRSRLIYVC-----PTPGSPCNS	137
PDX3 (At5g49970)	DEYEELTKQYSDGSVIPKPKNWGGFRLKPNLFEFWQGPS <b>RL</b> HDRLQ-YSLQDVNGNPAW	523
PNPO-RP (At2g46580)	EQSSQQVK--LDPSSGPVP-EYCLLLLEPEKVDYLNK---TNQRLFFSSMATGTGEKCW	191
PDX3 (At5g49970)	KIHLR <b>L</b> AP 530	
PNPO-RP (At2g46580)	TSEK <b>V</b> NP 198	

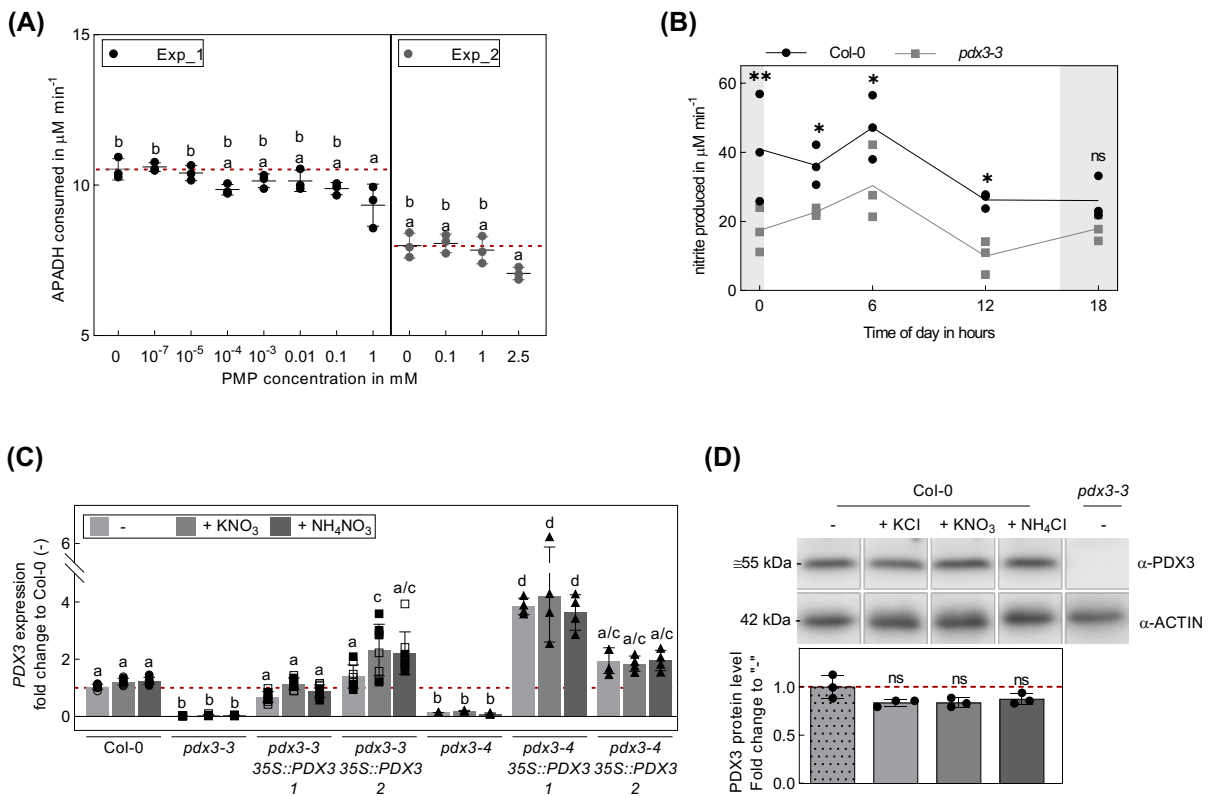
(B)



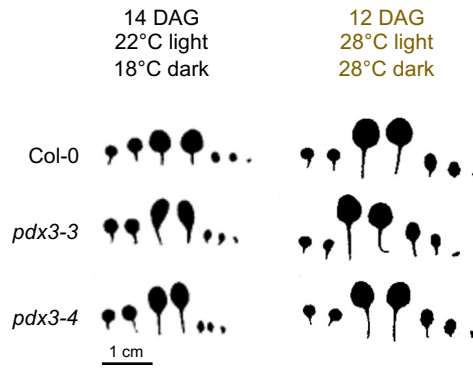
**Supplemental Figure S1. Probing the two-domain structure of PDX3.** (A) Amino acid sequence alignment of PDX3 (At5g49970, Uniprot: Q9LTX3) and the PDX3 related protein, PNP-RP (At2g46580, Uniprot: Q9ZPY1) described by Marbaix et al 2019. Similar residues are highlighted in gray and highly conserved residues of PDX3s and PNP-RPs as described in Marbaix *et al* 2019 are indicated with a gray arrow. The two PDX3 residues (D238 and R505) mutated in this study are indicated by a bold red font. (B) Immunochemical analysis of PDX3 protein levels in wild type (Col-0), *pdx3* and lines expressing either the *PDX3 D238A* or *PDX3 R505A* transgene. The analysis was performed on 14 days old plants grown on soil (unfertilized) under a 16 h photoperiod (120-160  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) at 22°C and 8 h darkness at 18°C using 20-25  $\mu\text{g}$  of total protein.



**Supplemental Figure S2. Phenotype of rosette leaves of *pdx3* compared to wild type.** Photographs of wild type (Col-0) and *pdx3* lines grown on unfertilized (-) and either potassium nitrate (+ KNO<sub>3</sub>), ammonium nitrate (+ NH<sub>4</sub>NO<sub>3</sub>) fertilized soil. The plants are 21 days old and were grown on soil under a 16 h photoperiod (120-160  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) at 22°C and 8 h darkness at 18°C and were watered either with water alone (-) or a 50 mM solution of the indicated compound every 9-10 days. The scale bar applies to all photographs. Individual images were digitally extracted for comparison.



**Supplemental Figure S3. Nitrate reductase activity and *PDX3* expression as a function of PMP and N fertilization, respectively.** (A) Activity of recombinant nitrate reductase in the presence of PMP, shown as rate of APADH (NADPH substitute) consumption in the presence of 0-2.5 mM PMP at pH 7.5 and 25°C. The data represents the mean  $\pm$  SD of three technical and two experimental replicates (Exp\_1 and Exp\_2). Statistical analysis was performed using ordinary one-way ANOVA with Sidak's multiple comparison test (different letters indicate  $p \leq 0.05$ ). (B) Nitrate reductase activity in rosette leaves of wild type and *pdx3-3* plants grown on unfertilized soil under a 16 h photoperiod ( $120-160 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) at 22°C and 8 h darkness harvested at 0 h (in the dark before the onset of light), 3 h, 6 h, 12 h (all in the light), and 18 h (2 h after onset of darkness). The data represents the mean  $\pm$  SD of 1 experimental and 3 biological replicates. Statistical analysis was performed using a two-tailed Student's unpaired *t*-test with Col-0 as control ( $^{ns}p > 0.05$ ,  $^*p \leq 0.05$ , and  $^{**}p \leq 0.005$ ). (C) Relative expression of *PDX3* in *pdx3* and complementing lines compared to wild type (Col-0) grown on unfertilized (-) and either potassium nitrate (+ KNO<sub>3</sub>) or ammonium nitrate (+ NH<sub>4</sub>NO<sub>3</sub>) fertilized soil. Data represents the mean  $\pm$  SD across 2 experimental replicates, (either open or filled symbols) except for *pdx3-4* and corresponding complementing lines, with 4 biological replicates each. Statistical analysis was performed using an ordinary one-way ANOVA with Tukey's multiple comparisons test for the transcript (different letters indicate  $p \leq 0.05$ ). (D) Protein levels of *PDX3* in wild type (Col-0) and *pdx3-3* (as a control) grown on unfertilized soil and wild type grown on either potassium chloride (+ KCl), potassium nitrate (+ KNO<sub>3</sub>) or ammonium chloride (+ NH<sub>4</sub>Cl) fertilized soil. Data represents the mean  $\pm$  SD of 3 biological replicates. Statistical analysis was performed using a two-tailed Student's unpaired *t*-test using condition (-) as control ( $^{ns}p > 0.05$ ). Plants were grown as in (B) and watered with water alone (-) or a 50 mM solution of the indicated compound every 9-10 days.



**Supplemental Figure S4.** Photograph of the leaves of wild type (Col-0) and *pdx3* lines grown up to 14 days after germination (DAG) under the standard temperature of 22°C compared to 12 DAG under 28°C. In these conditions and developmental stage, the number of true leaves (five) is equal. The scale bar applies to all photographs. Individual images were digitally extracted for comparison.

**Supplemental Table S1.** Oligonucleotides used in this study. Forward (F), reverse (R), left border (LB).

Purpose	Gene	AGI number	Sequence (5'-3' direction)	Notes	
Cloning	<i>PDX3</i>	At5g49970	F- CACCTGTGGTGTGGGAAGTCATT		
			R- AGGCCAAACCATCGTCTC		
Site-directed mutagenesis			F- CATTGTCTCTGTGGCTATTCCCTCTGGTTG	D238A	
			R- CAACCAGAGGGAATAGCCACAGAGACAATG		
			F- CAGGGACAGCCATCTGCTTTACATGACAGGC	R505A	
			R- GCCTGTCATGTAAAGCAGATGGCTGTCCCTG		
Genotyping			F- GCTTTGTCTACAGCGAACAAGG	<i>pdx3-3</i>	
			R- CGTCGTATAGAACATGCCTGC		
			LB- ATTTTGCCGATTTTCGGAAC	T-DNA	
			F- ACACACATAGATGTCCCTGGG	<i>pdx3-4</i>	
			R- AACCCAAACTTGGGTATCACTG		
			LB- ATATTGACCATCATACTCATTGC	T-DNA	
			<i>NahG</i>	F- GCCTTAGCACTGGA <del>ACT</del> CTG	<i>NahG</i>
				R- TCGGTGAACAGCACTTGCAC	
	<i>SID2</i>	At1g74710	F- GGTGCACCAGCTTTTATCGG	<i>sid2-1</i>	
			R- TGGAGTTGGATGCAGAGCAG		
<i>NPR1</i>	At1g64280	F- TGCTCTGCAATTGCTCTCCA	<i>npr1-2</i>		
		R- TGTTGCGGTCTTCACATTGC			
RT-qPCR	<i>ASN1</i>	At3g47340	F- GTGGCTTGTTTCGACTGCAAAG		
			R- TGAATCACAACCTCTTGACCCA		
	<i>ASN2</i>	At5g65010	F- CGACTGTACCAGGAGGTCCAA		
			R- TTCCATTCTTAGGAAGAGGATCT		
	<i>ATL31</i>	At5g27420	F- ACCGGTGGGCTTTTCTTAG	Pair 1	
			R- AACTGACGATGTTTCCTTACC		
			F- TGACCCGTATGCTTACAGCG	Pair 2 (N-supplementation)	
			R- CCTGCAGGAGTAACGCTACC		
	<i>GDH2</i>	At5g07440	F- CACTAACGCTCAAACCATGGC	Pair 1	
			R- GAACCACCAAGATCAATGGGC		
			F- GACTCGAGCCTTTCACAACATC	Pair 2 (N-supplementation)	
			R- AGCGAC TCGGTAACTCCAAG		
	<i>PR1</i>	At2g14610	F- ACACGTGCAATGGAGTTTGTG		

			R- TTGGCACATCCGAGTCTCACT	
<i>PDX3</i>	At5g49970		F- GGTAGTGAGTAGAAGTCATGGG	
			R- CGTTTACATGACAGGCTGCA	
<i>NIA1</i>	At1g77760		F- AGGCTACGCTTATTCTGGAGG	
			R- TGTTTCGGTTTCTCCTGGTGG	
<i>NIA2</i>	At1g37130		F- GTTTCATTGGTGGCCGGATG	
			R- TACCACCAACCTTCTTCGTCG	
<i>ACT2</i>	At3g18780		F- TTGTTCCAGCCCTCGTTTGT	
			R- CCTGGACCTGCCTCATCATACT	
<i>UBC21</i>	At5g25760		F- TAGCATTGATGGCTCATCCTGA	
			R- TTGTGCCATTGAATTGAACCC	