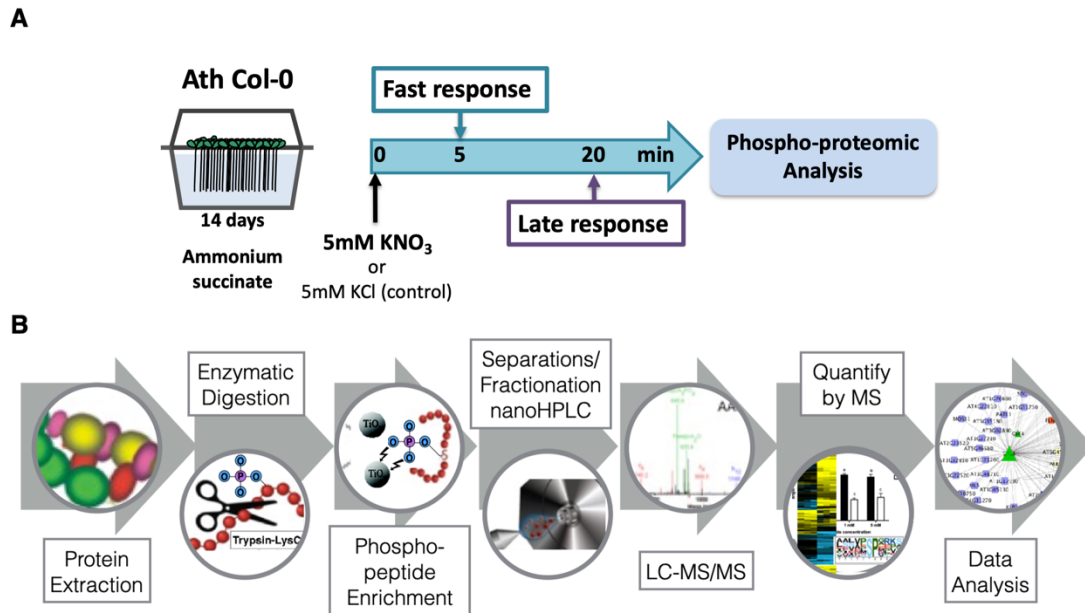


## Appendix

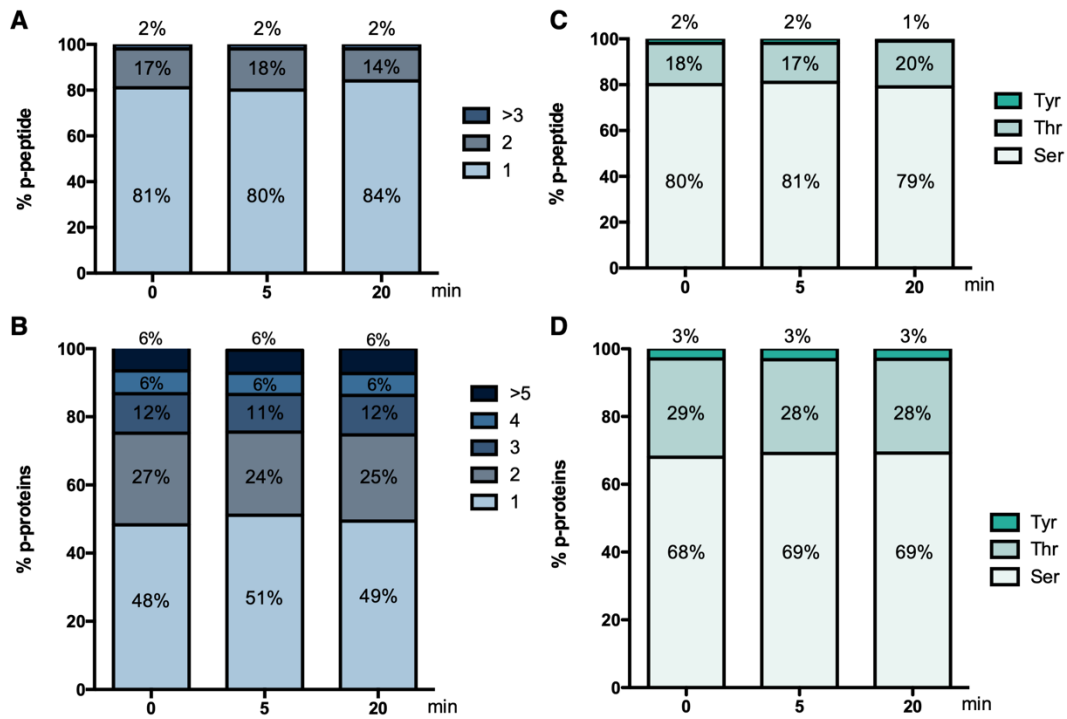
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**Appendix Figure S1. An overview of the root phosphoproteomic experiment.**

(A) Experimental design to identify changes in phosphoproteins in response to nitrate treatments in *Arabidopsis thaliana* roots.

(B) Phosphoproteomic strategy for the enrichment, quantification and analysis of phosphopeptides.



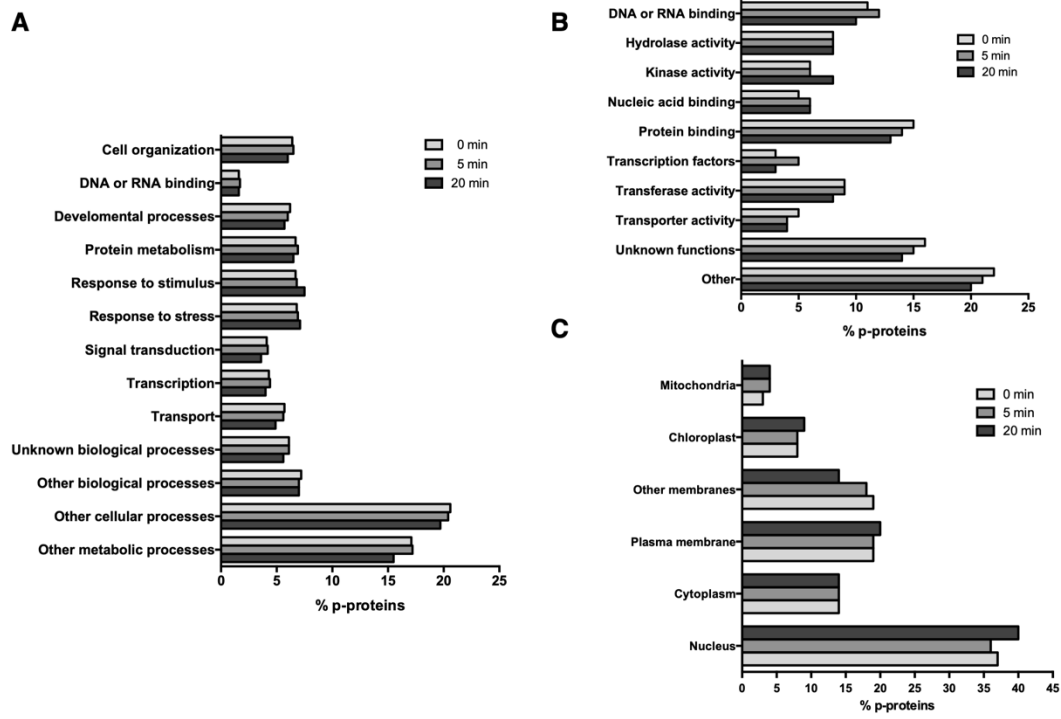
**Appendix Figure S2. An overview of the root phosphoproteomic analysis.**

(A) Distribution of the number of phosphosites per peptide for the indicated experimental dataset (0, 5 or 20 minutes after nitrate treatment).

(B) Relative distribution of phosphorylated residues in each peptide for indicated experimental dataset.

(C) Distribution of the number of phospho-peptides per protein for indicated experimental dataset.

(D) Percentage of phospho-proteins that present each phosphorylated residue for indicated dataset.

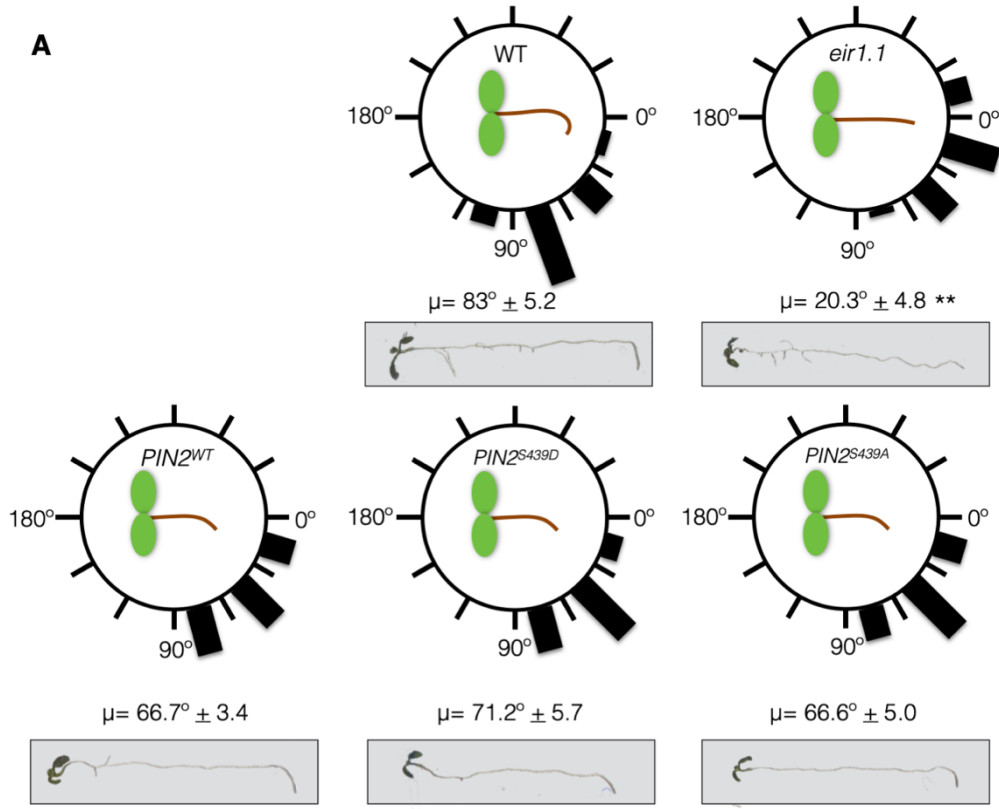


**Appendix Figure S3. Distribution of phosphoproteins across GO categories:** biological process (A), cellular functions (B) and sub cellular compartment (C) for each experimental dataset.

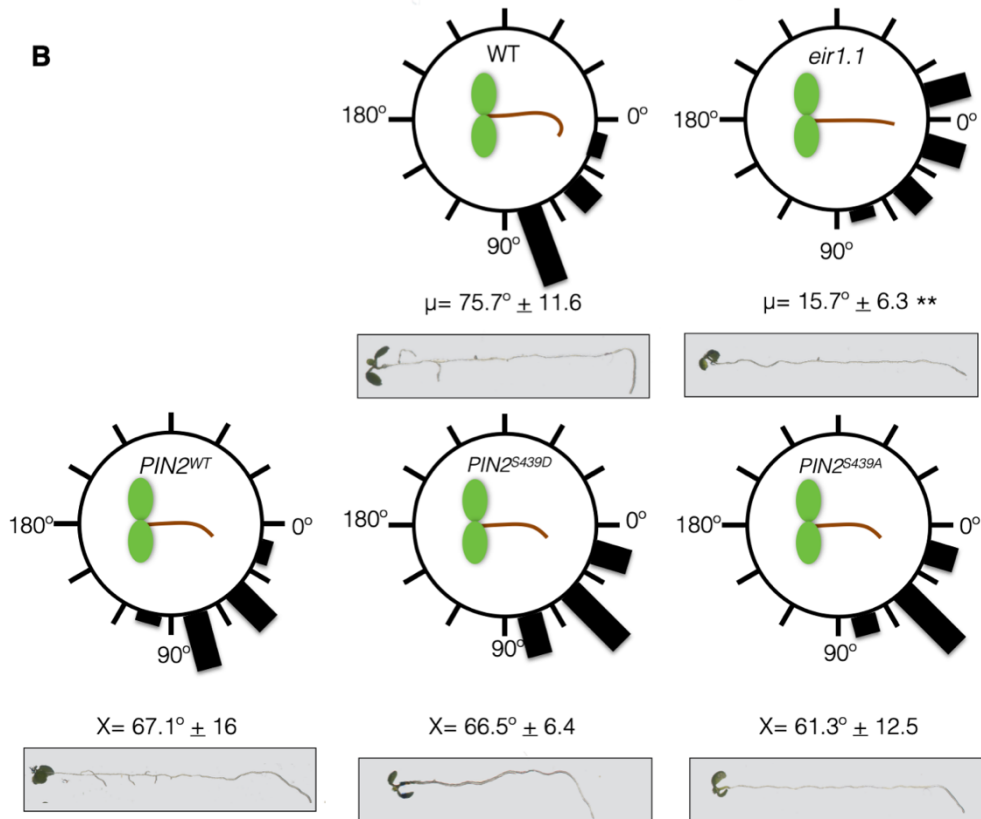


**Appendix Figure S4. Motif-X analysis of nitrate-responsive phosphopeptides.** Amino acid sequences from -5 to +5 residues (the phosphorylated residue was 0) were scanned with the Motif-X algorithm. Motif list of phosphopeptides that met the criteria for phosphoproteins up-regulated (A) and down-regulated (B) by nitrate at 5 min. Motif list of phosphopeptides that met the criteria for phosphoproteins up-regulated (C) and down-regulated (D) by nitrate at 20 min See Dataset EV2 for the complete set of nitrate-regulated phosphoproteins.

**A**



**B**



## Appendix Figure S5. Phosphoprotein PIN2 (S439) in root gravitropic response.

*pin2* null mutant background (*eir1-1*) complemented with non-modified or wild type version of PIN2 (PIN2<sup>wt</sup>), phospho-null (S439A) or phospho-mimic (S439D) versions of PIN2 (PIN2<sup>S439A</sup> or PIN2<sup>S439D</sup>, respectively) were grown in MS agar plate supplemented with 0.5 mM (A) or 5 mM (B) nitrate. At day 7, plates were rotated 90° and root curvature was measured at 24 hours with imageJ software. The figure shown a histogram of root gravitropic response where the length of black bars indicates the relative frequency of plants with the corresponding classes of angle. The average ( $\mu$ ) plus standard deviation of seedlings scored per line is indicated below each circle (3 independent biological replicate, 8-10 plants each experimental condition). Asterisk indicate statistically significant difference between means analyzed by unpaired, two-tailed and assuming equal variance *t-test* (\*\* $p < 0.01$ , ).

**Appendix Table S1. Over-represented Gene Ontology terms in response to nitrate at 5 min and 20 min.**

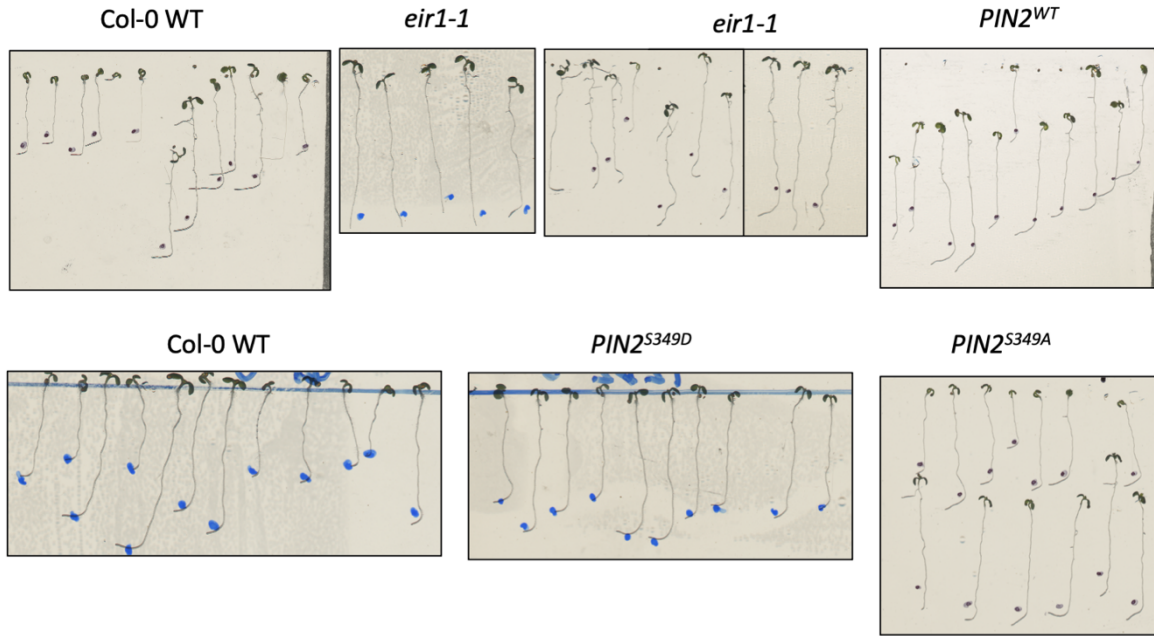
<b>Over-represented terms 5 min</b>	<b>p-value</b>
Efflux transmembrane transporter activity (GO:0015562)	4.88E-04
RNA binding (GO:0003723)	8.02E-03
Transcription coregulator activity (GO:0003712)	9.74E-03
Organic cyclic compound binding (GO:0097159)	2.52E-02
Nucleic acid binding (GO:0003676)	3.85E-02
Binding (GO:0005488)	3.98E-02
Protein phosphatase binding (GO:0019903)	4.16E-02
Phosphatase regulator activity (GO:0019208)	4.16E-02
<b>Over-represented terms 20 min</b>	<b>p-value</b>
Protein phosphatase regulator activity (GO:0019888)	2.25E-04
Protein phosphatase binding (GO:0019903)	2.56E-04
Hydrolase activity (GO:0016788)	3.23E-02
Protein binding (GO:0051879)	4.08E-02
Transferase activity (GO:0016740)	4.75E-02
Binding (GO:0005488)	4.90E-02



**Source Data for Appendix Figure S5.**

Representative pictures for biological replicates from PIN2 root gravitropic experiments at 0.5 mM (A) or 5 mM (B) nitrate.

**A**



**B**

