Expanded View Figures

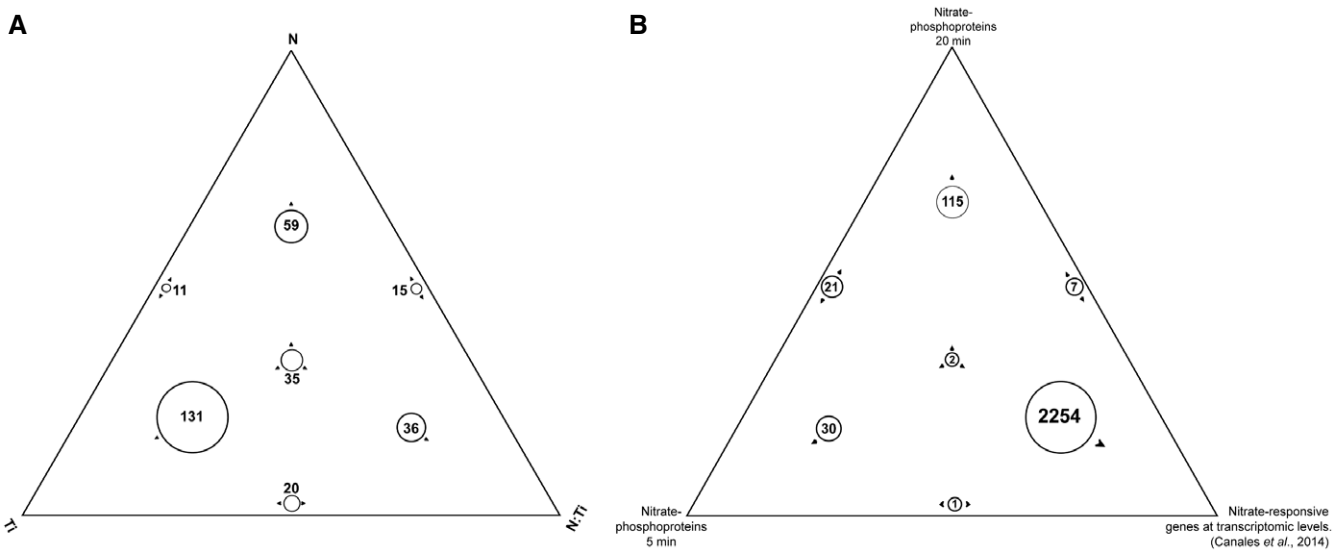

Figure EV1. Phosphoproteins affected by nitrate response.

A The list of phosphoproteins with significant factors achieved by two-way ANOVA was represented by the Sungear tool. The Sungear figure was represented by a triangle that shows the factors of ANOVA model at the vertices [nitrate treatment (N), time (Ti), and the interaction between treatment and genotype (N–Ti)]. The circles inside the triangle represent the phosphoproteins with statistical changes by the different factors ($P < 0.05$), as indicated by the arrows around the circles. The size of each circle was represented proportional to the number of phosphoproteins associated with that circle. The number of phosphoproteins in each circle is shown inside or next to the corresponding circle.

B The list of encoded genes in response to nitrate at transcriptomic (dataset 27 affymetrix experiment Canales *et al*, 2014) and phosphoproteomic (our work) levels were compared and represented using the Sungear tool. The triangle shows each dataset at the vertices: transcriptomic dataset, phosphoproteomic data (5 min), and phosphoproteomic data (20 min). The position and the arrows of the circle indicated which vertices or anchor the data belong. The largest circles are on the perimeter that indicate most of the data are associated with only one dataset.

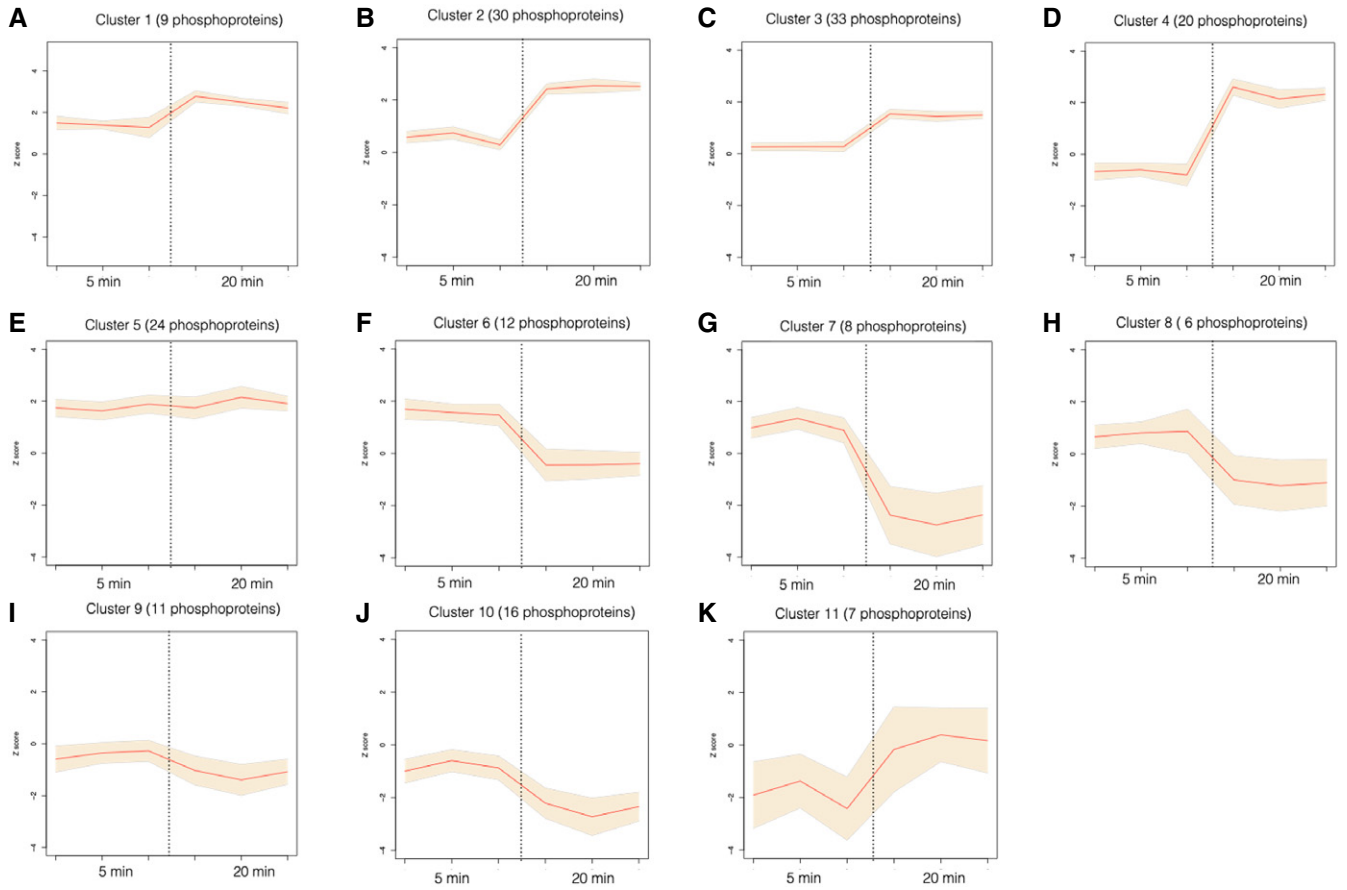

Figure EV2. Hierarchical clustering of nitrate phosphoproteins with differential abundance at 5 or 20 min in response to nitrate treatments.

A–K Nitrate phosphoprotein abundance patterns for eleven clusters in response to nitrate at 5 or 20 min (correlation distance threshold < 0.9) as described in Figure 2. Graphs show mean values of Z-scored normalized phosphoproteins levels (orange line) and 95% confidence interval for mean values of each Cluster for the independent experiments (shadow).

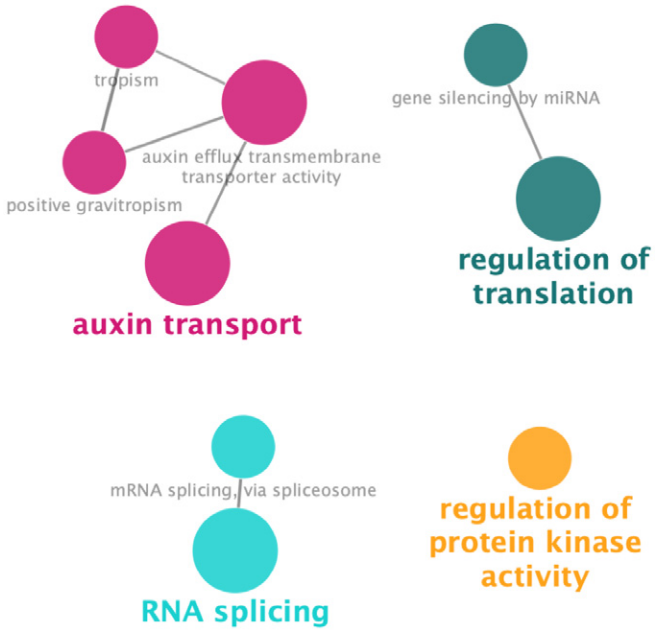

Figure EV3. Over-represented biological processes in the phosphoproteins gene network in response to nitrate (hypergeometric test with FDR, $P < 0.05$).

The network was analyzed using BINGO and ClueGO tools in Cytoscape software. Over-represented gene ontology terms are shown as a node connected by edges based on semantic relationships as defined in the ontology.

Source data are available online for this figure.

Figure EV4. PIN2 protein analysis in response to nitrate.

A PIN2 detection by Phos-tag Western blotting (source data Fig 5C). *Arabidopsis* plants (Col-0) were grown in ammonium as only nitrogen source and treated with 5 mM KNO_3 or 5mM KCl as control. Total protein from roots was analyzed in SDS–PAGE using Phos-tag to detect changes in phosphorylation status. Immunoblotting was performed with PIN2 antibody. Total proteins isolated from *eir1.1* roots were used as a negative control. PIN2-specific signal was tested as control in Col-0 and *eir1-4* mutant by normal Western blot SDS–PAGE.

B Phos-tag Western blotting analysis to detect PIN2 phosphorylation (two independent biological replicates). *Arabidopsis* plants (Col-0) were grown in ammonium as only nitrogen source and treated with 5 mM KNO_3 or 5 mM KCl, as a control condition. Total proteins from roots were analyzed in SDS–PAGE using Phos-tag to detect changes in phosphorylation status. Immunoblotting was performed with PIN2 antibody. Blue and red asterisks indicate a slow- or fast-mobility band corresponding to a more or less phosphorylated PIN2, respectively. The numbers below the blot correspond to relative quantification by densitometry using Image J of these two bands, normalized by loading control.

C PIN2-GFP detection by SDS–PAGE Western blotting. Quantification of two independent replicate Western blot experiments against PIN2-GFP protein comparing nitrate-treated (KNO_3) and control (KCl) condition at 5 min in *Arabidopsis* roots for all genotypes *eir1-1* mutant background was complemented with PIN2::$PIN2^{wt}$-GFP ($PIN2^{wt}$), PIN2::$PIN2^{S439D}$-GFP ($PIN2^{S439D}$ phospho-mimic point mutation), or PIN2::$PIN2^{S439A}$-GFP ($PIN2^{S439A}$, phospho-null point mutation). Total proteins isolated from Col-0 roots were used as a negative control. Bars represent the mean plus standard deviation of 2 biological replicates.

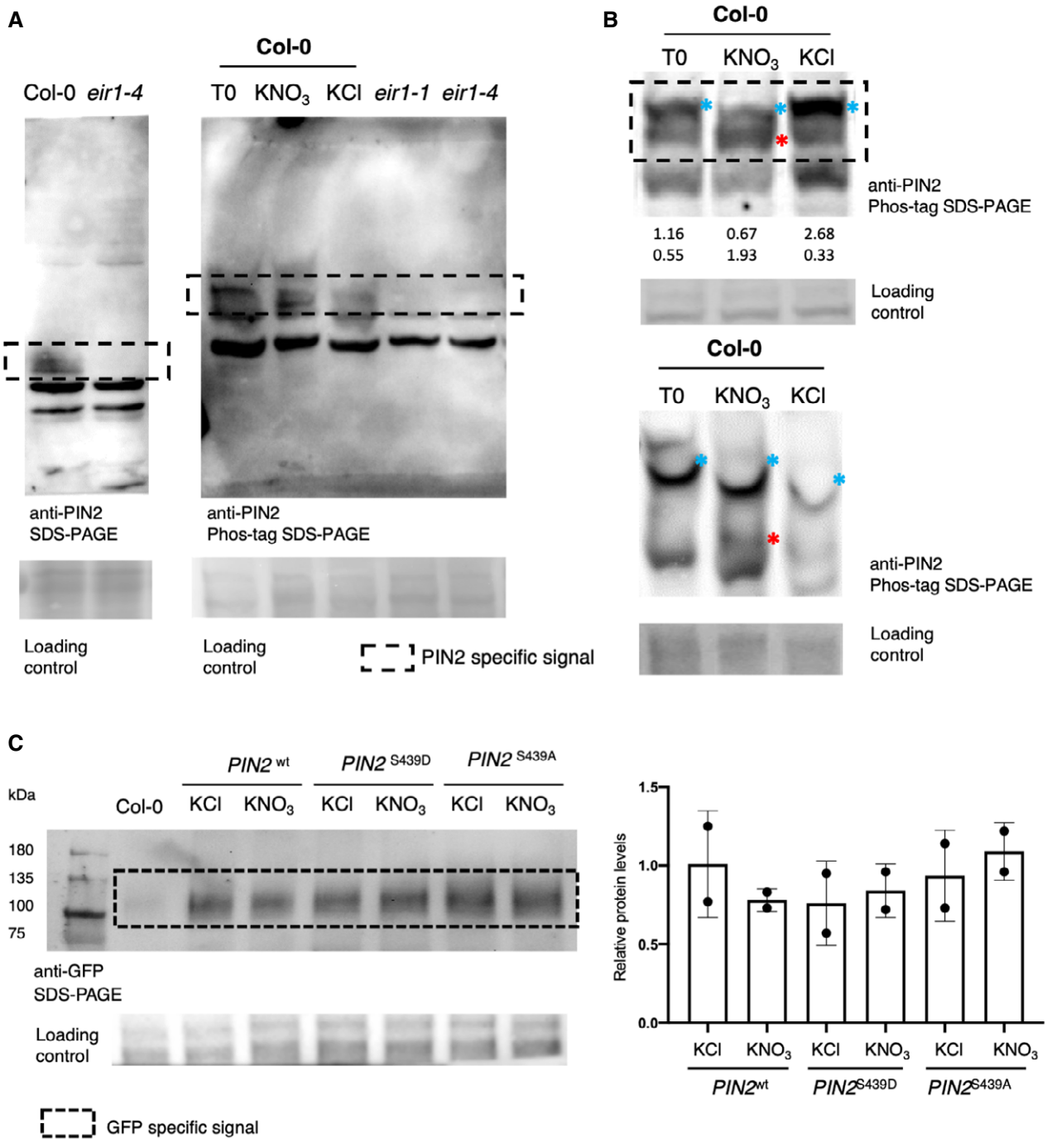
A
Col-0
Col-0 eir1-4
T0 KNO3 KCl eir1-1 eir1-4
anti-PIN2
SDS-PAGE
anti-PIN2
Phos-tag SDS-PAGE
Loading
control
Loading
control
PIN2 specific signal
B
Col-0
T0 KNO3 KCl
anti-PIN2
Phos-tag SDS-PAGE
1.16 0.67 2.68
0.55 1.93 0.33
Loading
control
Col-0
T0 KNO3 KCl
anti-PIN2
Phos-tag SDS-PAGE
Loading
control
C
kDa
Col-0
PIN2 wt
PIN2 S439D
PIN2 S439A
KCl KNO3 KCl KNO3 KCl KNO3
180
135
100
75
anti-GFP
SDS-PAGE
Loading
control
GFP specific signal
Relative protein levels
1.5
1.0
0.5
0.0
KCl KNO3 KCl KNO3 KCl KNO3
PIN2wt
PIN2S439D
PIN2S439A

Figure EV4.