Supporting Information

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SI Methods

Isolation of Root Cells. *Arabidopsis* lines that express the GFP protein in epidermis, cortex, endodermis, pericycle, and stele cells were grown as described in *Methods* and then treated with 5 mM KNO_3 or KCl. Roots were harvested after 2 h of treatment, and protoplasts were extracted by incubating the roots on the cell wall-digesting enzymes cellulysin (Calbiochem; catalog no. 219466) and pectolyase (Sigma-Aldrich; catalog no. P3026), as described previously (1). GFP-positive cells were separated on a FACSAria II cell sorter (BD Biosciences) directly into lysis buffer from an miRVana microRNA (miRNA) isolation kit (Ambion; catalog no. AM1560).

Extraction of RNA from Root Tips. WT Col-0 plants were grown in ammonium succinate for 2 wk and then treated with 5 mM KNO_3 or 5 mM KCl for 2 h. Plants were maintained on RNAlater RNA stabilization reagent (Qiagen; catalog no. 76106) while meristematic and elongation zones were excised from 20 roots. RNA was extracted from mersitematic and elongation zones using the miRVana miRNA isolation kit.

RNA Isolation and Quantitative RT-PCR. RNA was isolated from whole roots using TRIzol reagent (Invitrogen; catalog no. 15596026) or from root cells using the miRVana miRNA isolation kit according to the manufacturer's instructions. cDNA synthesis was carried out using Improm-II reverse transcriptase (Promega; catalog no. 28692236) according to the manufacturer's instructions. Quantitative RT-PCR (qRT-PCR) was performed using Brilliant SYBR Green QPCR reagent (Agilent; catalog no. 600546) on a Stratagene MX3000P qPCR system. Data were normalized by expression of the clathrin adaptor complex medium subunit family protein gene *At4g24550* (2, 3).

1. Gifford ML, Dean A, Gutiérrez RA, Coruzzi GM, Birnbaum KD (2008) Cell-specific nitrogen responses mediate developmental plasticity. *Proc Natl Acad Sci USA* 105(2): 803–808.
2. Aceituno FF, Moseyko N, Rhee SY, Gutiérrez RA (2008) The rules of gene expression in plants: Organ identity and gene body methylation are key factors for regulation of gene expression in *Arabidopsis thaliana*. *BMC Genomics* 9(1):438.
3. Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible W-R (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiol* 139(1):5–17.
4. Wang R, Xing X, Crawford N (2007) Nitrite acts as a transcriptome signal at micromolar concentrations in *Arabidopsis* roots. *Plant Physiol* 145(4):1735–1745.
5. Malamy JE, Benfey PN (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* 124(1):33–44.

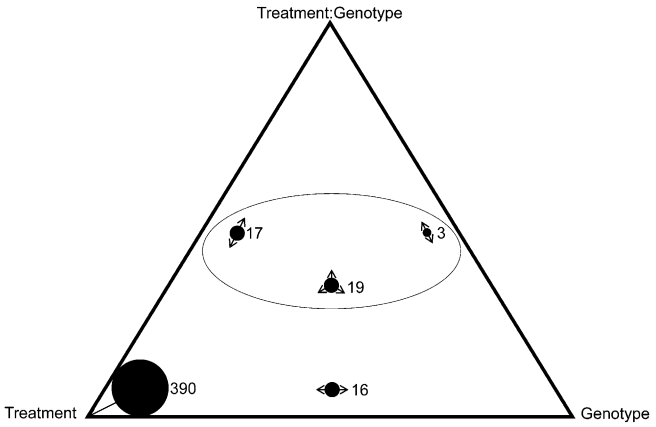

Fig. S1. Sungear analysis of genes with significant treatment (T), genotype (G), and treatment:genotype (TG) interaction factors reveals a specific role for *AFB3* in the root nitrate response. The list of genes with significant factors obtained by two-way ANOVA of Affymetrix data are represented in a Venn diagram using the VirtualPlant tool Sungear (http://www.virtualplant.org). The Sungear triangle shows the factors (T, G, and TG) at the vertices (anchors). The circles inside the triangle (vessels) represent the genes controlled by the different factors, as indicated by the arrows around the vessels. The area of each vessel (size) is proportional to the number of genes associated with that vessel. The number of genes in the vessel is shown next to the corresponding vessel. The group of 39 genes with significant TG factors is highlighted.

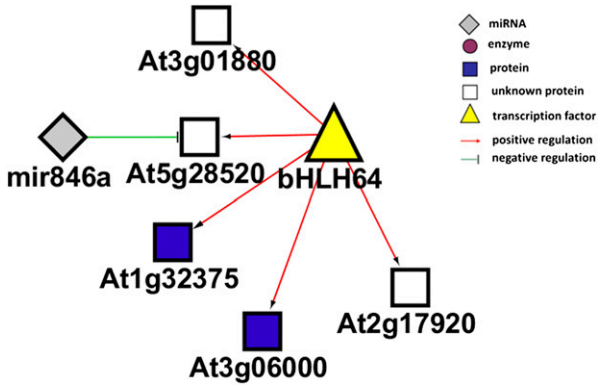

Fig. S2. Small network of genes with a significant TG factor. A network view of our list of genes with altered nitrate response in *afb3-1* was generated using the Gene Networks tool available at the VirtualPlant Web page (www.virtualplant.org). The analysis generated two networks; the small network controlled by the bHLH64 transcription factor is shown. The nodes represent genes (gray squares, miRNA; purple circles, enzyme-coding genes; blue squares, protein-coding genes; white squares, unknown protein-coding genes; yellow triangles, transcription factor-coding genes), and the edges represent miRNA/*TARGET* post-transcriptional regulation or predicted regulatory interactions based on the occurrence of a transcription factor-binding site on the promoter of the gene. Green edges represent repression, and red edges represent induction based on correlation analysis of our Affymetrix data ($|x|>0.7$).

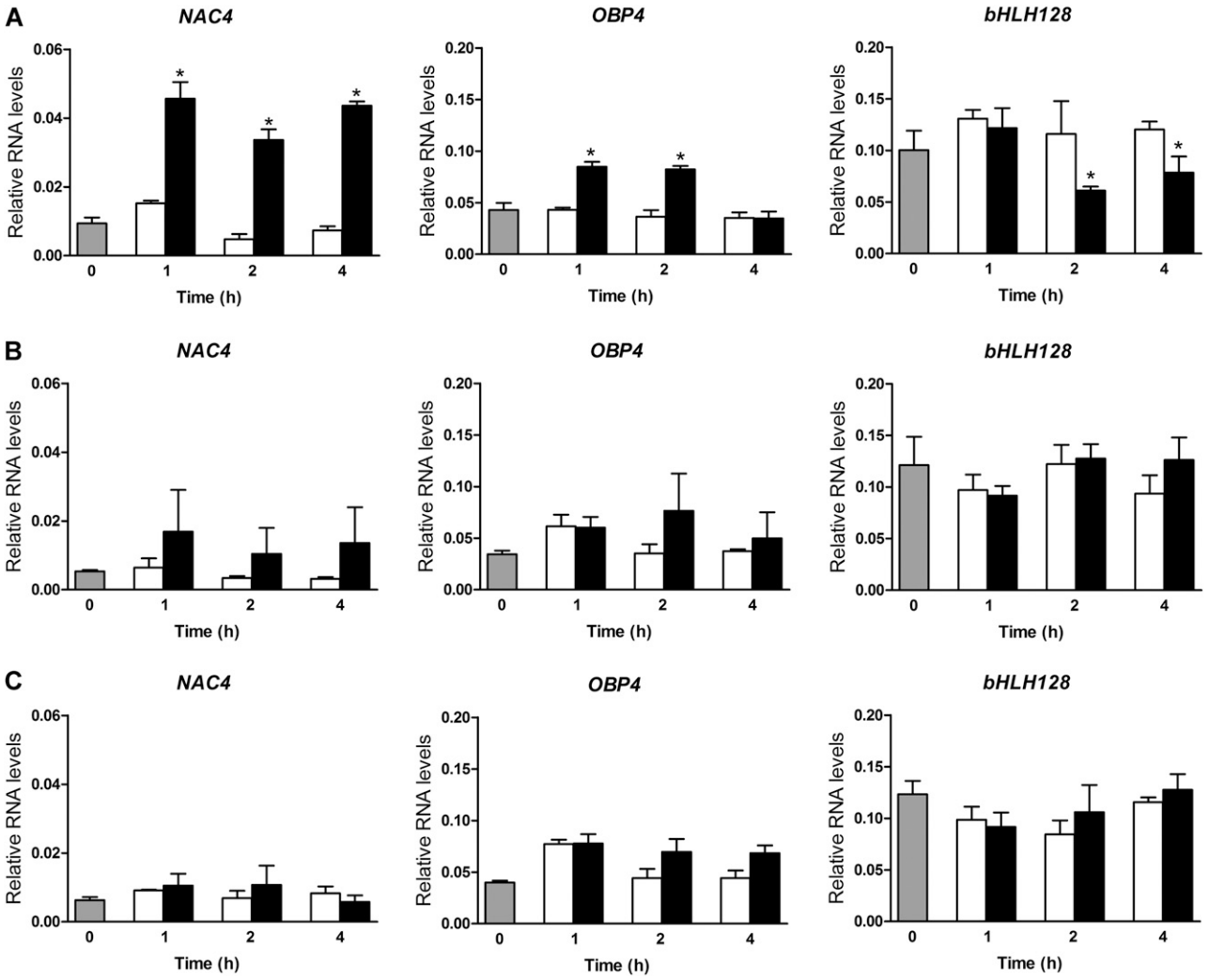

Fig. S3. *NAC4*, *OBP4*, and *bHLH128* respond directly to nitrate as a signal. (*A*) Nitrate reductase-null plants (*nia1/nia2*) (4) were grown in ammonium succinate for 2 wk and then treated with 5 mM KNO_3 or 5 mM KCl for 1, 2, and 4 h. RNA levels of the *NAC4*, *OBP4*, and bHLH128 transcription factors were measured using qRT-PCR. Gray bars represent time 0 (before treatment), white bars represent KCl treatment, and black bars represent KNO_3 treatment. (*B*) WT Col-0 plants were grown as described above and then treated with 250 μM KNO_2 or 250 μM KCl as control for 1, 2, and 4 h. This concentration was chosen based on results of concentration-dependent experiment reported by Wang et al. (4), who showed that 250 μM KNO_2 caused peak induction of *NITRATE REDUCTASE 1* (*NIA1*) and *NITRITE REDUCTASE* (*NIR*). In addition, this concentration did not produce an increase in NO concentration, indicating that 250 μM is a proper concentration for evaluating specific nitrite response. RNA levels of the *NAC4* and *OBP4* transcription factors were measured by qRT-PCR. Gray bars represent time 0 (before treatment), white bars represent KCl treatment, and black bars represent KNO_2 treatment. (*C*) WT Col-0 plants were grown as described above and treated with 5 mM NH_4Cl or 5 mM KCl as control for 1, 2, and 4 h. RNA levels of the *NAC4* and the *OBP4* transcription factors were measured by qRT-PCR. Gray bars represent time 0 (before treatment), white bars represent KCl treatment, and black bars represent NH_4Cl treatment. In *A*–*C*, data are mean ± SE of three biological replicates. Asterisks denote means with statistically significant differences between controls and treatments ($P < 0.05$).

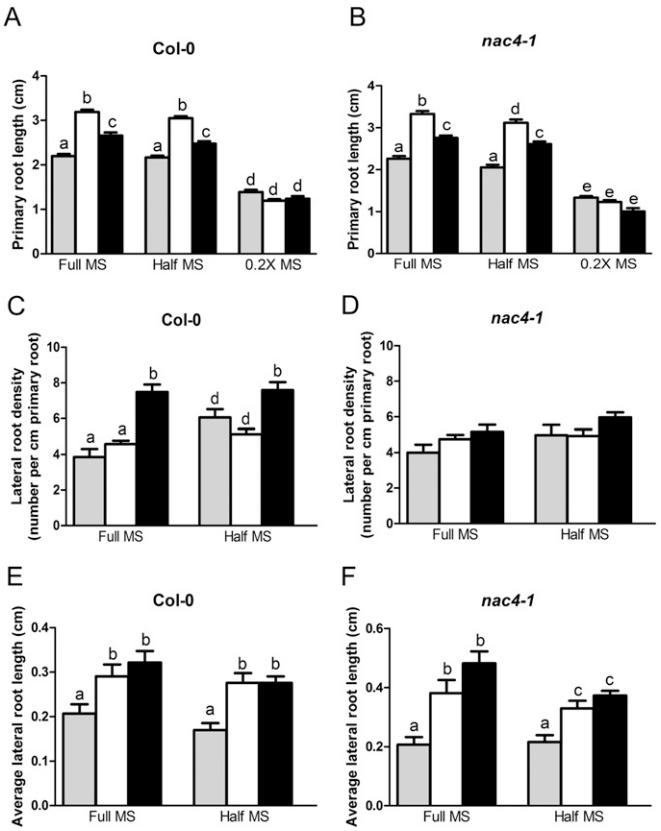

Fig. S4. Primary root length, lateral root density, and lateral root length of plants grown in diluted MS medium without N (MS –N medium). WT Col-0 (*A*, *C*, and *E*) and *nac4-1* mutant plants (*B*, *D*, and *F*) were grown in full-strength MS –N, half-strength MS –N, and 0.2× MS –N supplemented with 0.5 mM ammonium succinate for 2 wk and then treated (*C* and *D*) with 5 mM KNO_3 (black bars) or 5 mM KCl (white bars) for 3 d. Primary root length (*A* and *B*) and lateral root length (*E* and *F*) were measured using ImageJ. For lateral root measurements (*C* and *D*), initiating and emerging lateral roots were counted using differential interference contrast (DIC) optics. Gray bars represent plants at day 0, white bars represent plants after 3 d of KCl treatment, and black bars represent plants after 3 d of KNO_3 treatment. Values are mean ± SE of three biological replicates ($n = 30$). Different letters represent means with statistically significant differences ($P < 0.05$).

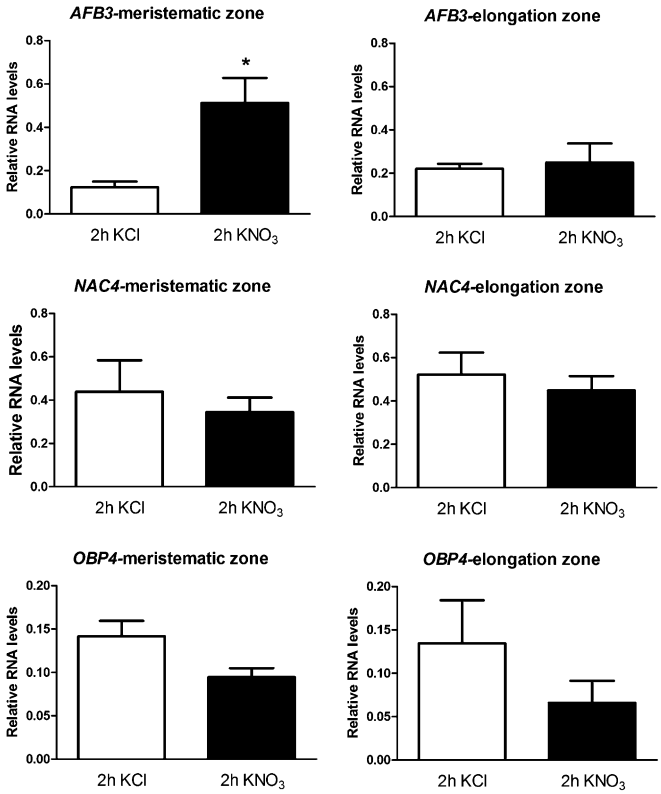

Fig. S5. Nitrate regulation of *AFB3*, *NAC4*, and *OBP4* in meristematic and elongation zones of the primary roots. WT Col-0 plants were grown in ammonium succinate for 2 wk and then treated with 5 mM KNO_3 or 5 mM KCl for 2 h. Plants were maintained on RNAlater RNA stabilization reagent while meristematic and elongation zones were excised from 20 roots. RNA was extracted from mersitematic and elongation zones using the miRVana miRNA isolation kit. RNA levels of *AFB3*, *NAC4*, and *OBP4* were measured using qRT-PCR. Data are mean ± SE of three biological replicates. White bars represent KCl treatment, and black bars represent KNO_3 treatment. Asterisks denote means with statistically significant differences between the KCl and KNO_3 treatments ($P < 0.05$).

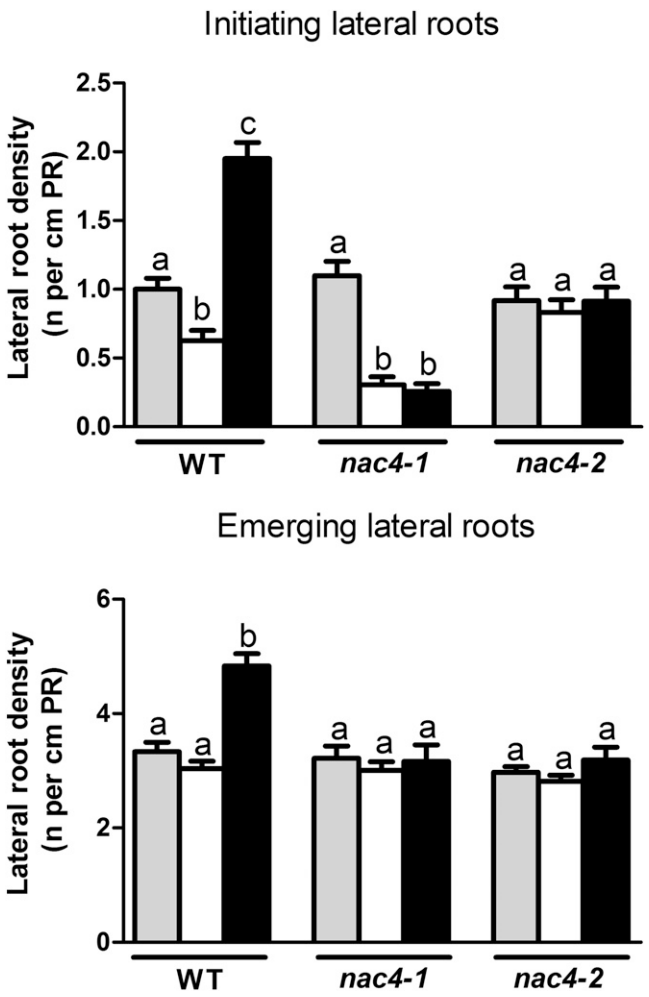

Fig. S6. The increases in initiating and emerging lateral root density triggered by nitrate are altered in the *nac4* mutants. The initiating [stage I, II, III, IV, Va, Vb, Via, Vib, and VII (5)] and emerging lateral roots of Col-0 WT plants and *nac4-1* and *nac4-2* mutant plants at day 0 (gray bars), treated for 3 d with 5 mM KCl (white bars) or 5 mM KNO_3 (black bars), were counted using DIC optics. Values are mean ± SE of three biological replicates ($n = 15$). Different letters represent means with statistically significant differrences ($P < 0.05$).

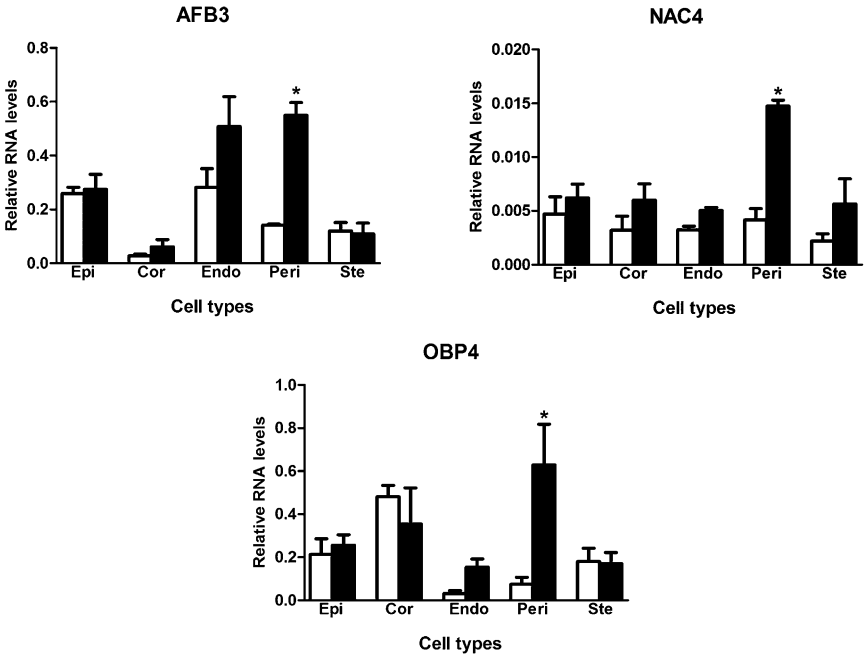

Fig. S7. Expression of *NAC4* and *OBP4* is regulated by nitrate in pericycle cells. *Arabidopsis* lines expressing GFP in epidermis (epi; pWER:GFP), cortex (cor; pAt1g09750:GFP), endodermis (endo; pSCR:GFP), pericycle (E3754; from Scott Poethig enhancer trap lines), and stele (ste; pWOL:GFP) were treated for 2 h with 5 mM KNO_3 or KCl. Protoplasts were prepared from roots, and pericycle cells expressing GFP were sorted by fluorescence-activated cell sorting. RNA levels for *NAC4* and *OBP4* were measured by qRT-PCR. Values are mean ± SE of three biological replicates. White bars represent KCl treatment, and black bars represent KNO_3 treatment. Asterisks denote means with statistically significant differences between control and treatment ($P < 0.05$).

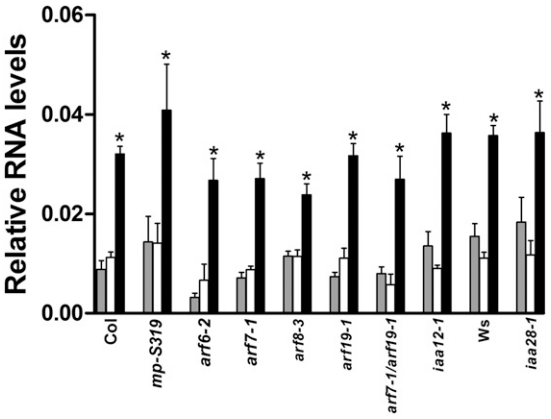

Fig. S8. *NAC4* regulation in Aux/IAA and ARF mutants. WT Col-0 or Ws and ARF and Aux/IAA mutant plants were grown in ammonium succinate for 2 wk and then treated with 5 mM KNO_3 (black bars) or 5 mM KCl (white bars) for 2 h. RNA levels of the *NAC4* transcription factor were measured by qRT-PCR. Values are mean ± SE of three biological replicates. The gray bars represent time 0 (before treatment), white bars represent KCl treatment, and black bars represent KNO_3 treatment. Asterisks denote means with statistically significant differences between control and treatment ($P < 0.05$).

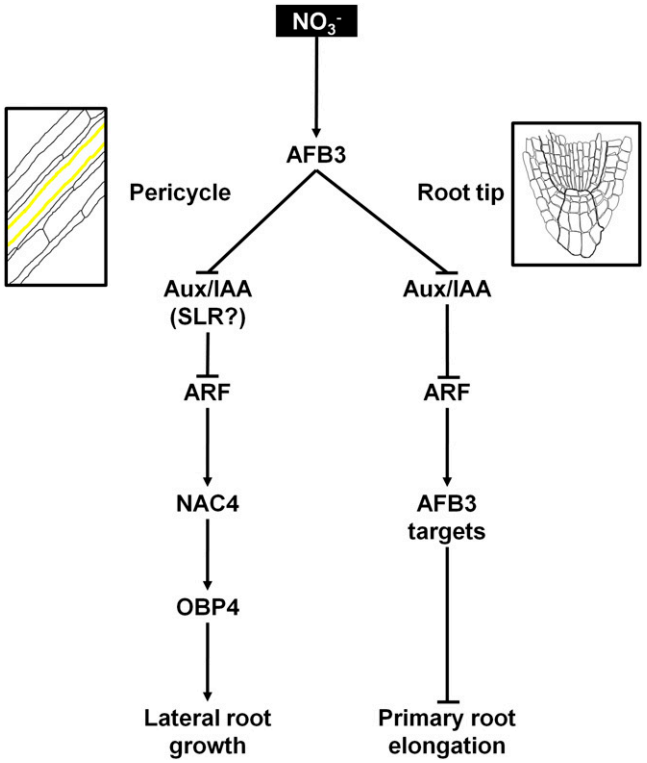

Fig. S9. Model of nitrate regulation of root system architecture by AFB3. Nitrate induction of AFB3 activates two different pathways to control primary and lateral root growth. In the pericycle, AFB3-mediated degradation of SLR/IAA14 or other Aux/IAA activates transcription of *NAC4* by an unidentified ARF. Induction of *NAC4* leads to induction of *OBP4*. This can occur through direct or indirect action of *NAC4* over *OBP4*. Induction of AFB3-NAC4-OBP4 induces lateral root growth. In primary root tips, AFB3 regulates an unidentified Aux/IAA-ARF module that represses primary root growth in response to nitrate. Given that *AFB3* induction is specific to the meristematic zone, this is likely related to inhibition of cell division.