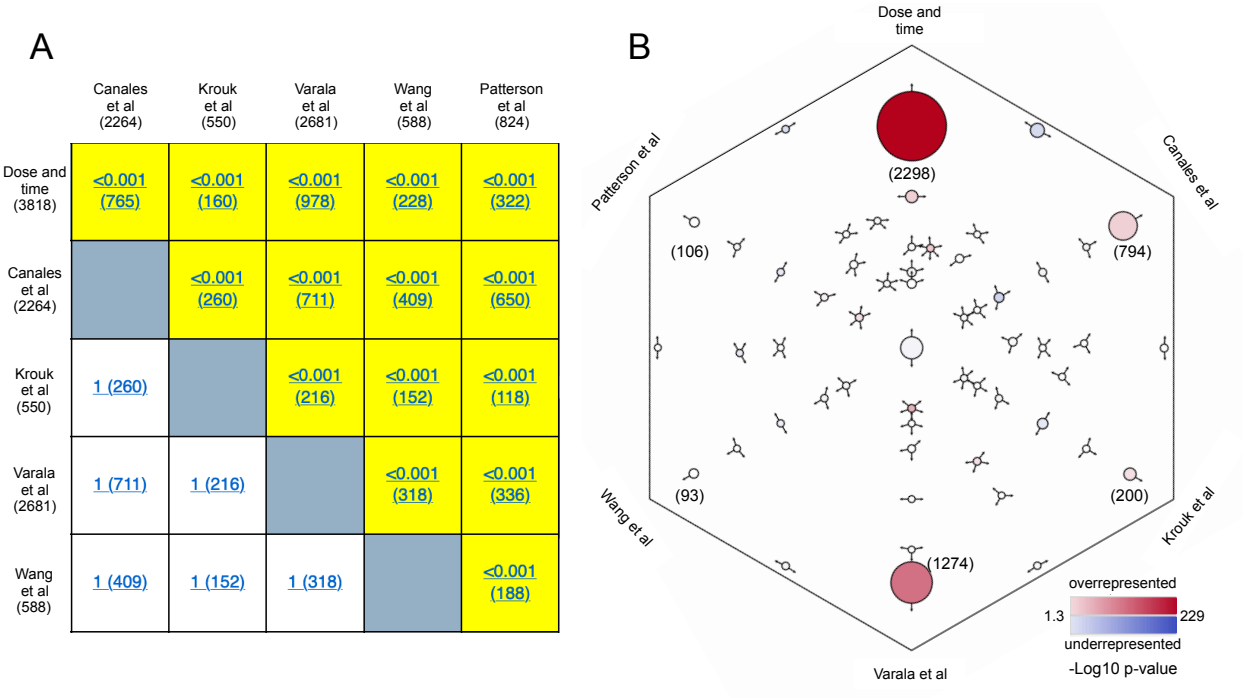
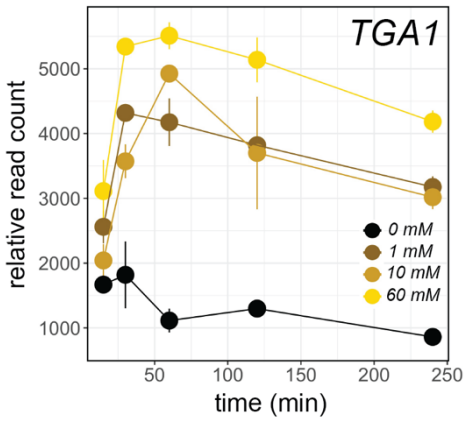


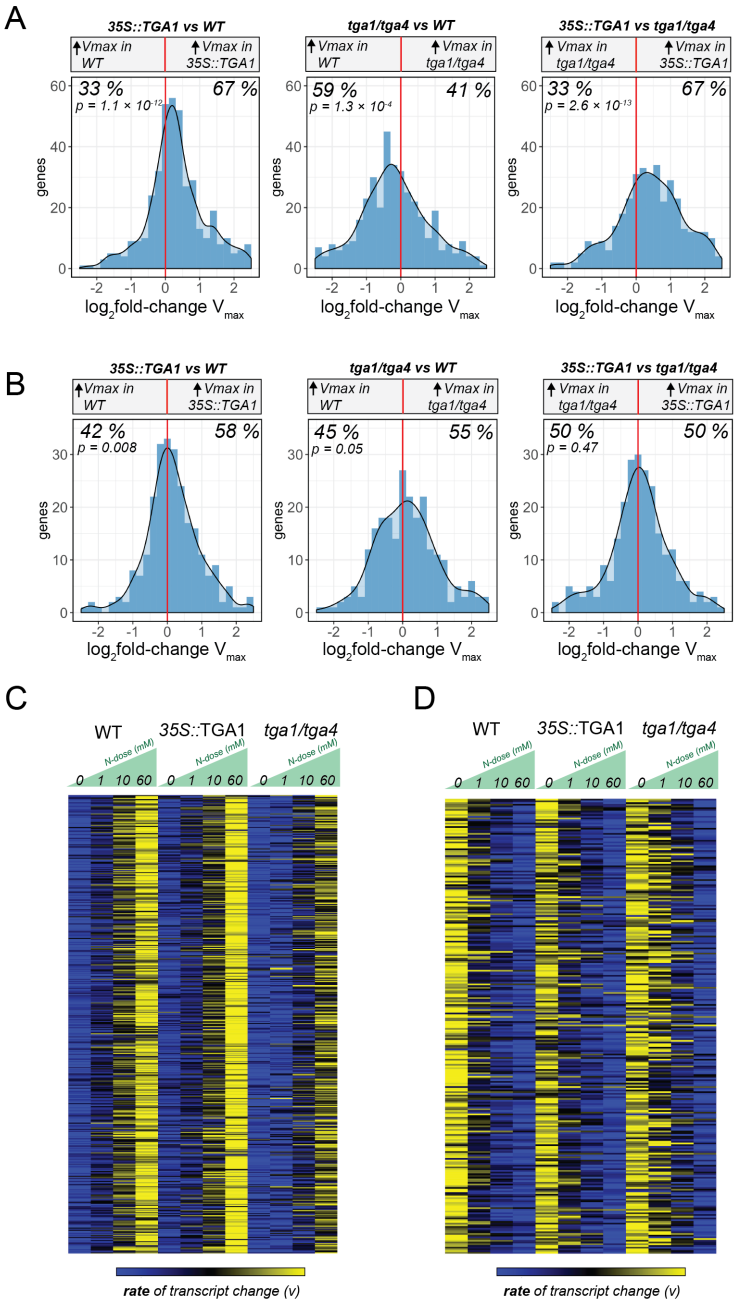
Supplementary Figure 1 **– Assay of N-dose responses over time captures known and novel N-regulated genes. A:** Overlap of N-dose responsive genes found in this study (response to N-dose over time) captures N-responsive genes found in previous studies. *Canales et al. 2014* (*1*), is a list of consistently regulated genes by N; *Krouk et al. 2010* (*2*) is a list of very early N-responsive genes; *Varala et al. 2018* (*3*) is a list of N-regulated genes in a fine-scale time series; *Patterson et al. 2010* (*4*) is a list of genes regulated by ammonium; and *Wang et al. 2004* (*5*)is a list of regulated genes by nitrate and not by downstream metabolites. The display uses the 'GeneSect' function in VirtualPlant (*6*) to calculate the significance of the gene intersects, where *Arabidopsis* genome was used as background. Significant intersections are colored yellow, with the number of genes found in the intersect indicated. **B**: Common or unique genes regulated by N in each experiment using 'Sungear' analysis (*6*). Each vertex of the hexagon represents an experiment, and circles represent the number of genes that are unique to each set (numbers in parentheses) or shared between particular experiments, indicated by the arrow around the vessels. Significance of under or over-represented intersections between experiments is estimated using a binomial test.



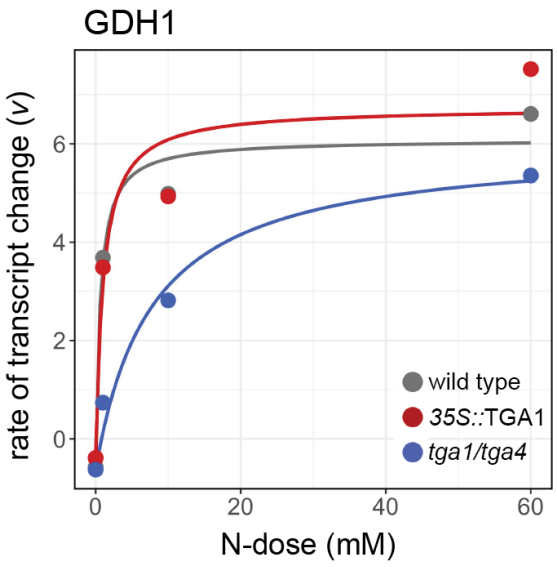
Supplementary Figure 2 **– TGA1 expression in roots is affected by N-dose over time.**
Normalized gene expression levels of TGA1 over time under different N-doses.

	Stele (366)	Epidermis/ Cortex (2164)	Lateral root cap (5111)	Pericycle (5112)	Michaelis-Menten Modeled (1153)
Endodermis/ Pericycle (720)	<0.001 (203)	<0.001 (360)	<0.001 (483)	<0.001 (447)	<0.001 (50)
Stele (366)		<0.001 (196)	<0.001 (205)	<0.001 (129)	<0.001 (31)
Epidermis/ Cortex (2164)	1 (196)		<0.001 (1841)	<0.001 (1843)	0.921 (69)
Lateral root cap (5111)	1 (205)	1 (1841)		<0.001 (4679)	0.062 (214)
Pericycle (5112)	1 (129)	1 (1843)	1 (4679)		0.266 (203)

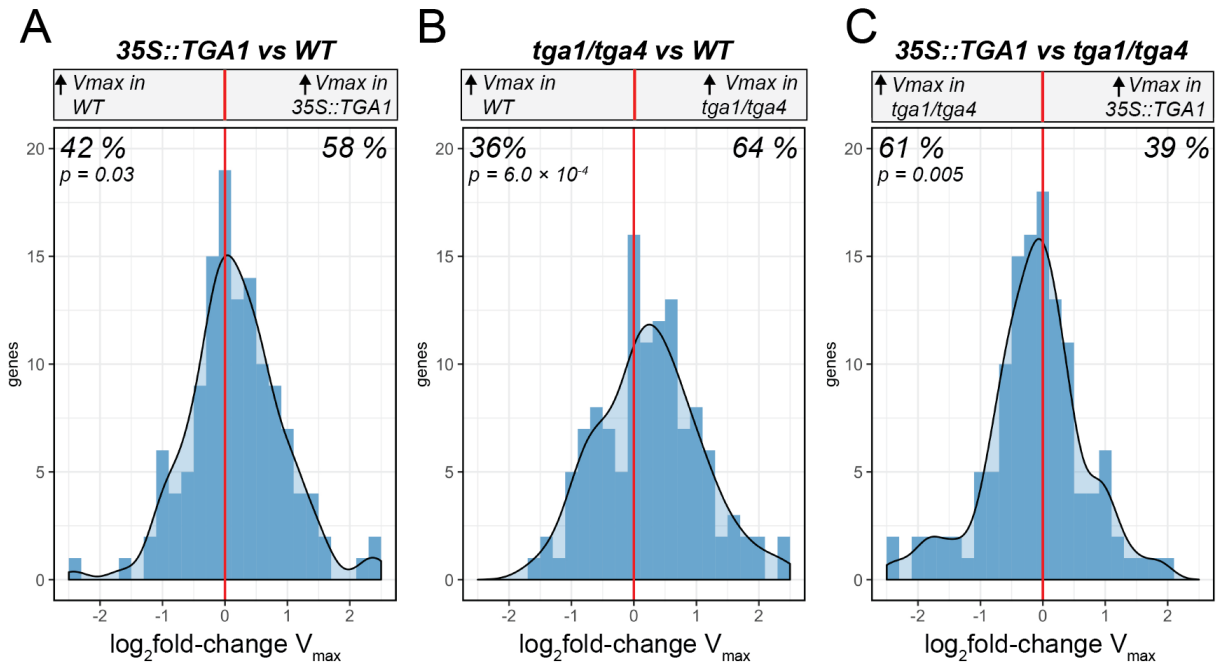
Supplementary Figure 3 **– Genes modeled by Michaelis-Menten (MM) kinetics in whole roots are enriched in N responses that occur within the inner cell-types of the root.** Cell-type specific responses to N, as reported in (*7*) were overlapped with N-responsive genes modeled by MM kinetics. We used the 'GeneSect' function in VirtualPlant (*6*) to calculate the significance of the gene intersects, where *Arabidopsis* genome was used as background. Significant intersections are colored yellow, with the number of genes found in the intersect indicated.



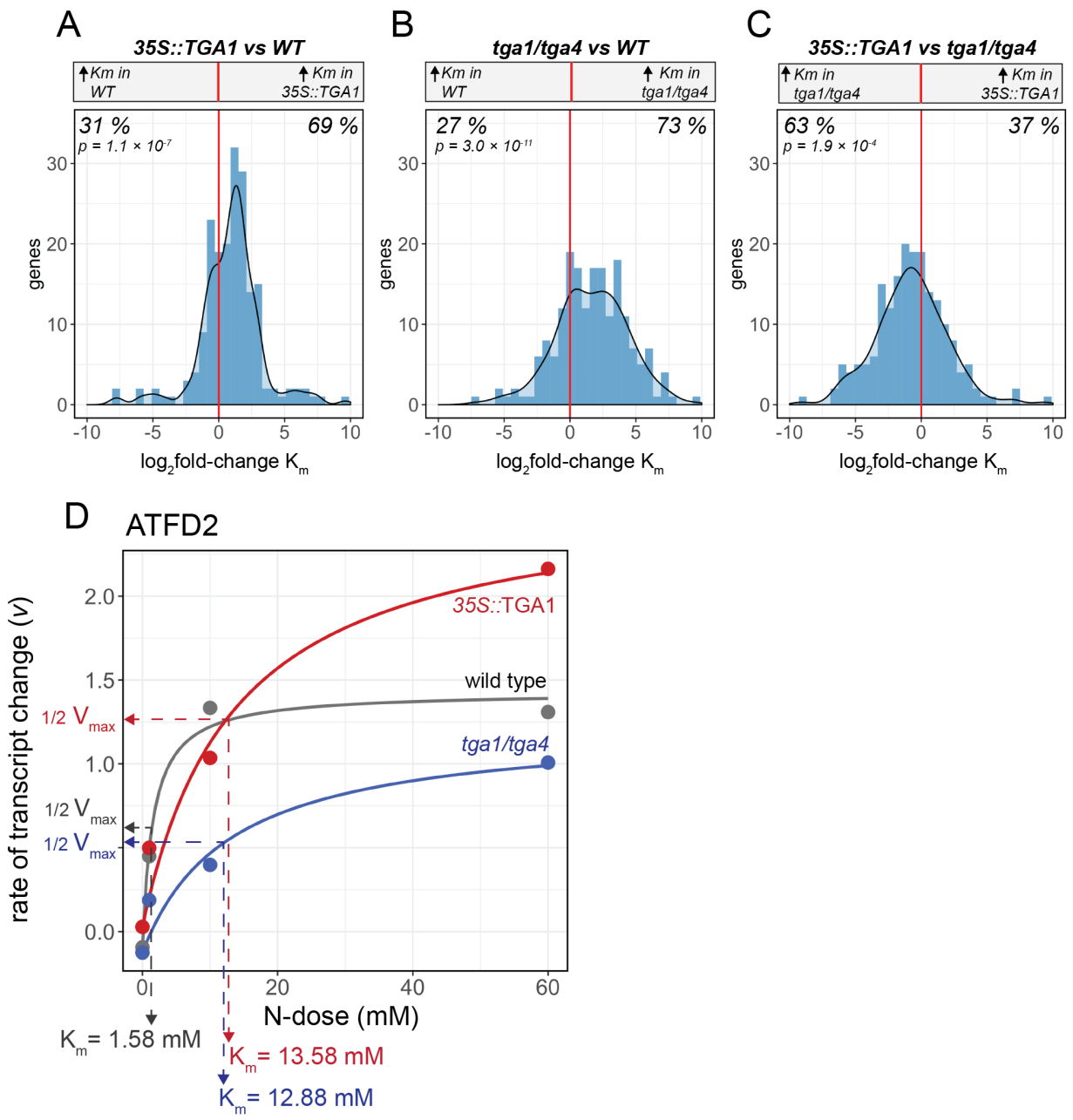
Supplementary Figure 4 **– V_{max} comparison for all N-dose responsive genes in roots, without MM significance threshold.** (**A**) Comparison of V_{max} of 446 genes upregulated by N across genotypes. For these 446 genes, a histogram of the $\log_2$ fold changes of V_{max} between *35S::*TGA1 vs. wild-type, *tga1/tga4* vs. wild-type and *35S::*TGA1 vs. *tga1/tga4* are shown. A bias towards higher or lower change in V_{max} was assessed (binomial test). (**B**) as (**A**) for 266 downregulated genes. (**C**) heatmap of (**A**). (**D**) heatmap of (**B**).



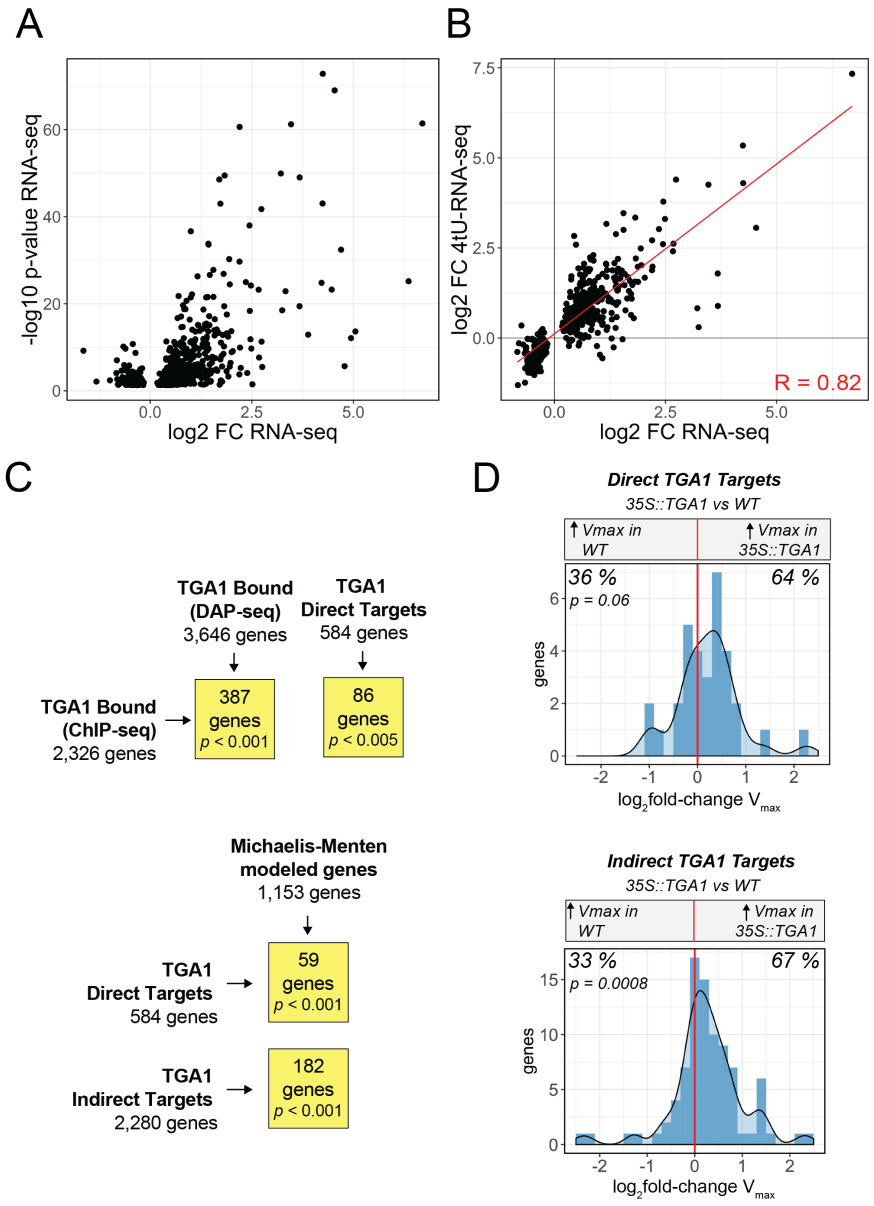
Supplementary Figure 5 **– Altering TGA1 expression *in planta* has an effect on N-dose dependent V_{max} levels of GDH1 mRNA in roots.** In wild-type, *35S::*TGA1 and *tga1/tga4* lines, changes in rate of transcriptional change of GDH1 are fit by the MM model.



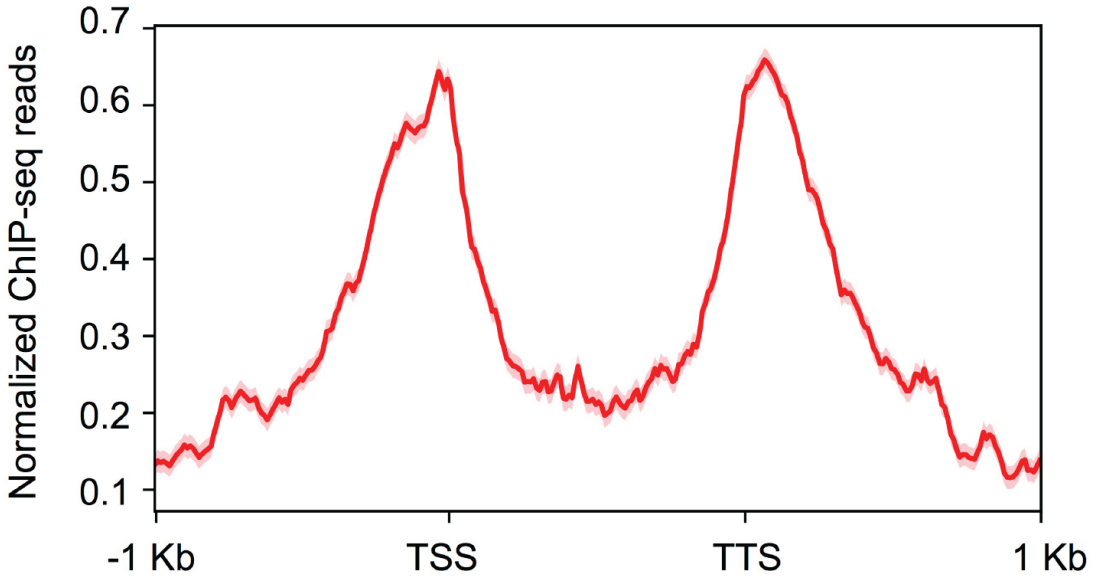
Supplementary Figure 6 **– Changes in TGA1 levels *in planta* affects the maximum rate of N-dose responsive gene repression (V_{max}).** V_{max} of 132 genes downregulated by N across genotypes which were fit significantly by the Michaelis-Menten model ($p < 0.05$). For these 132 genes, a histogram of the $\log_2$ fold changes of V_{max} between (**A**) *35S::*TGA1 and wild-type (**B**), *tga1/tga4* and (**C**) wild-type and *35S::*TGA1 and *tga1/tga4* are shown. A bias towards higher or lower change in V_{max} was assessed (binomial test). We note for repressed genes, higher V_{max} values indicates greater rates of transcript repression.



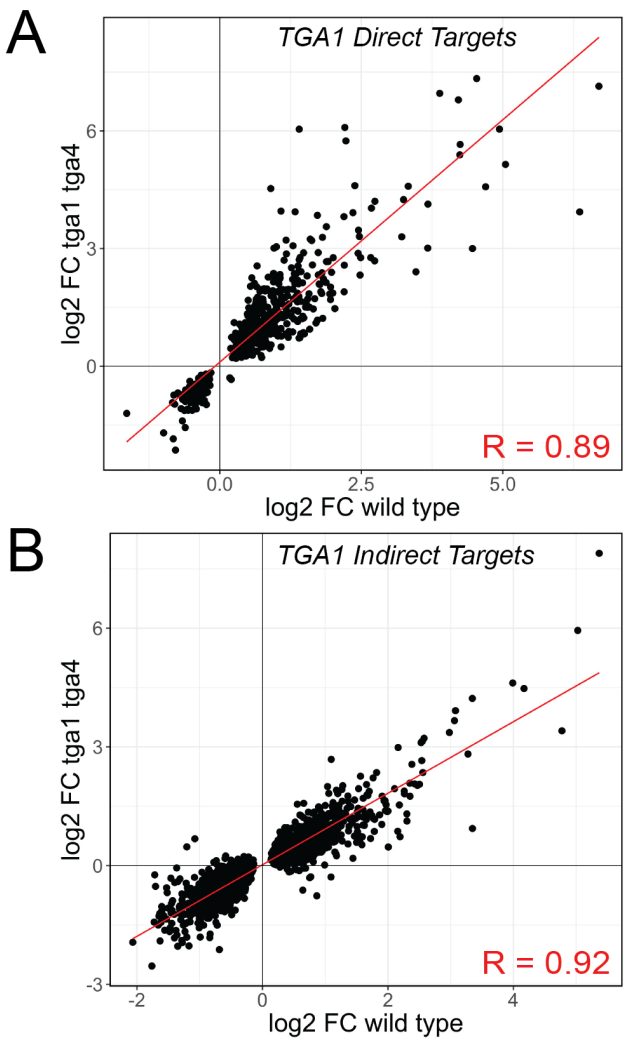
Supplementary Figure 7 **– Changes in TGA1 levels *in planta* affects the K_m of N-dose responsive genes. A - C:** Comparison of fold-change differences in K_m of 192 genes upregulated by N-dose across all three genotypes (tga1/4 and *35S::*TGA1 and wild-type) were fit significantly by the Michaelis-Menten model ($p < 0.05$). For these 192 genes, histograms of the $\log_2$ fold changes of K_m between (**A**) *35S::*TGA1 vs. wild-type, (**B**) *tga1/tga4* vs. wild-type and (**C**) *35S::*TGA1 vs. *tga1/tga4* are shown. A bias towards higher or lower change in K_m was assessed (binomial test). (**D**) An example gene ATDF2 (ferredoxin-like superfamily protein), illustrates how K_m values can increase when TGA1 is over-expressed (*35S::*TGA1), as well as when it is absent (*tga1/tga4*).



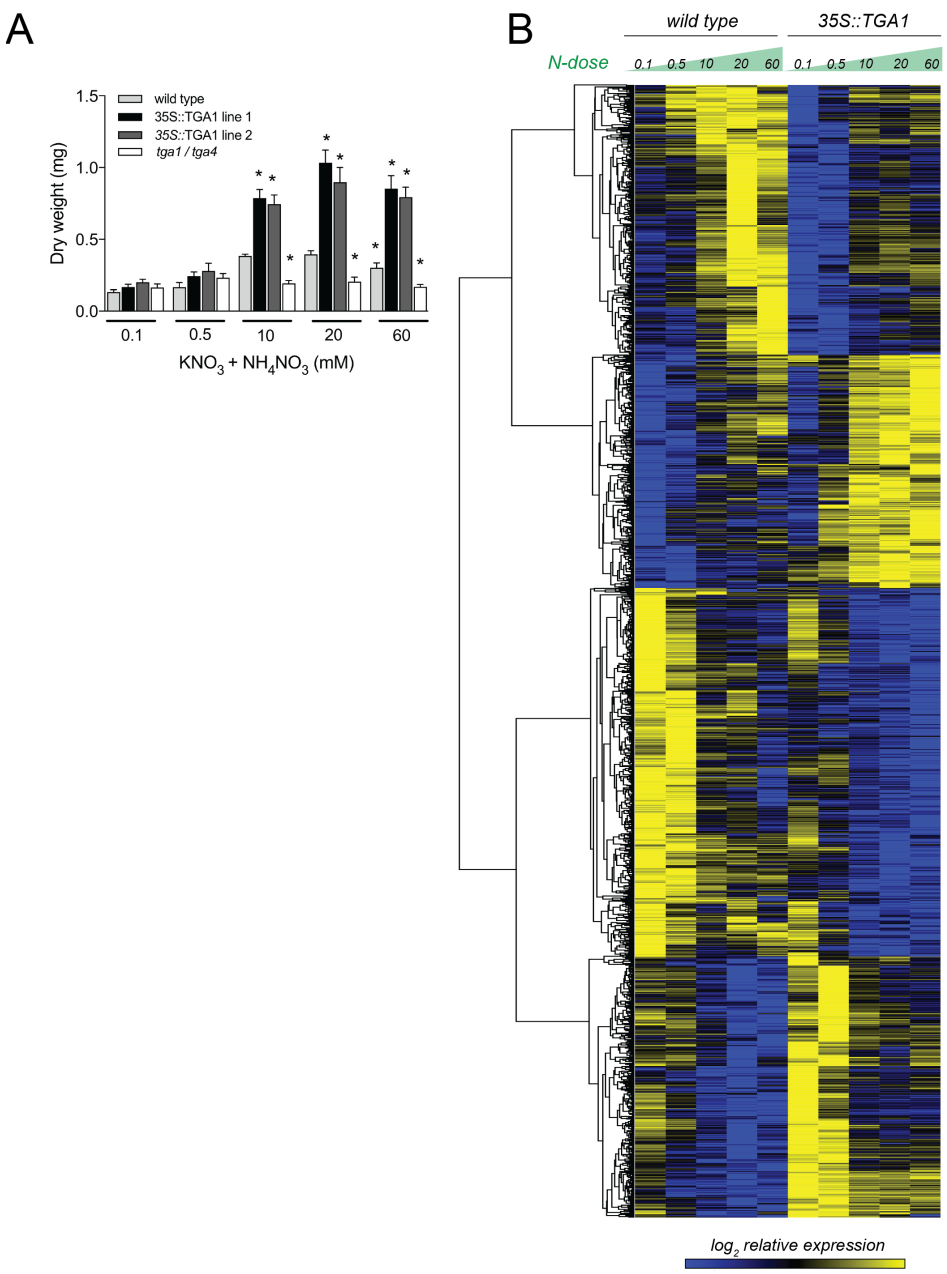
Supplementary Figure 8 **– TARGET TF-perturbation assay in root cells detects TGA1-dependent active transcription that overlaps with N-dose dependent MM modeled genes *in planta*. A:** Both the magnitude of differential expression and corresponding significance value of genes directly regulated by TGA1 in root cells. **B**: Correlation analysis of the levels of differential expression direct targets of TGA1 found through RNA-seq of steady state mRNA or 4tU-RNA-seq of *de novo* mRNA (Pearson $p < 0.05$). **C**: TGA1-bound target genes captured by ChIP-seq in root cells are enriched in: (*i*) TGA1 targets as found by DAP-seq (*8*) and (*ii*) genes directly regulated by TGA1 within the TARGET experiment (Monte Carlo test). Genes directly or indirectly regulated by TGA1 are enriched in MM modeled N-dose responsive genes. **D**: V_{max} of genes modeled by MM captured as directly (34 genes) or indirectly (93 genes) regulated by TGA1.



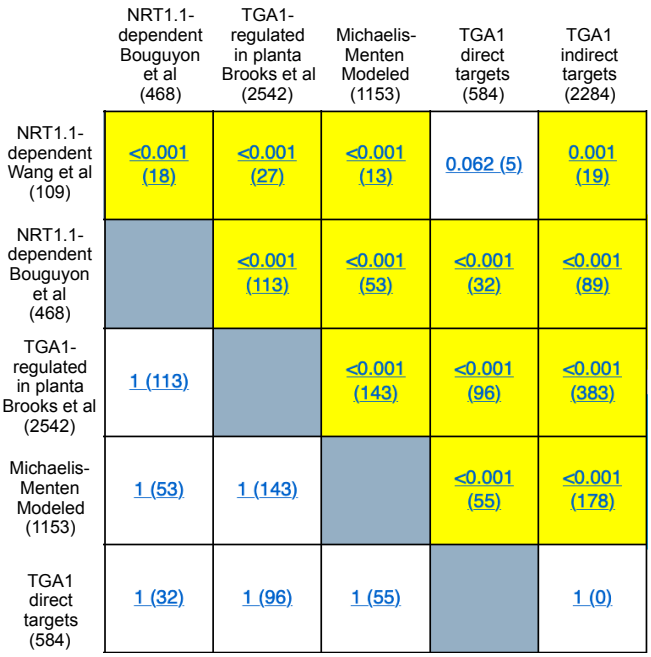
Supplementary Figure 9 **– TGA1 binding to gene targets in root cells captured by ChIP-seq in TARGET assay occurs close to the transcription start site (TSS) and transcription termination site (TTS).** The number of normalized ChIP-seq reads mapping within the 1000-bp upstream region of the TSS to the 1000-bp downstream region of TTS.



Supplementary Figure 10 **– Regulation of TGA1 direct and indirect targets genes in root cells does not change in the absence of TGA4.** The TARGET TF-perturbation assay was performed in root cells isolated from either the wild-type or the *tga1/tga4* mutant background. Levels of differential expression in *tga1/tga4* double mutant plants significantly correlate with levels of differential expression in wild-type plants for both TGA1 direct targets (**A**) and indirect targets (**B**) (Pearson $p < 0.05$).



Supplementary Figure 11 **– Overexpression of TGA1 (*35S::*TGA1) *in planta* impacts the N-dose response at the level of root transcriptome and growth rate. A:** Total biomass responses to N-dose for wild-type, *tga1/tga4,* and two independent *35S::*TGA1 transgenic lines (* $p < 0.05$, t-test). **B:** Transcriptomic analysis of wild-type and *35S::*TGA1 lines grown under steady state conditions. The N-dose response of 1,398 genes were perturbed by over-expression of TGA1 (2-way ANCOVA $p < 0.05$).



Supplementary Figure 12 **– Overlap of gene target lists indicate that NRT1.1 and TGA1 are part of the same N-dose signaling pathway that regulates genes whose response follows Michaelis-Menten kinetics.** NRT1.1-dependent genes captured by two independent studies (Wang et al. 2009; Bouguyon et al. 2015) (*9, 10*) overlap significantly with TGA1-regulated genes in roots *in planta* (Brooks et al 2019) (*11*), TGA1-regulated genes captured in root cells by TARGET, and genes in roots that follow MM kinetics (this study).

Supplementary Table 1 – List of genes fit by multivariate linear model.
Supplementary Table 2 – List of genes fit by Michaelis-Menten model.
Supplementary Table 3 – List of GO Terms of genes fit by Michaelis-Menten model.
Supplementary Table 4 – List of genes found by TARGET-RNA-seq and ChIP-seq assays.
Supplementary Table 5 – List of gene GO Terms found by TARGET-RNA-seq assay.
Supplementary Table 6 – List of genes differentially expressed under steady state conditions.

Supplementary References

1. J. Canales, T. C. Moyano, E. Villarroel, R. A. Gutierrez, Systems analysis of transcriptome data provides new hypotheses about Arabidopsis root response to nitrate treatments. *Frontiers in Plant Science* **5**, (2014).
2. G. Krouk, P. Mirowski, Y. LeCun, D. E. Shasha, G. M. Coruzzi, Predictive network modeling of the high-resolution dynamic plant transcriptome in response to nitrate. *Genome Biol* **11**, R123 (2010).
3. K. Varala *et al.*, Temporal transcriptional logic of dynamic regulatory networks underlying nitrogen signaling and use in plants. *Proc Natl Acad Sci U S A* **115**, 6494-6499 (2018).
4. K. Patterson *et al.*, Distinct signalling pathways and transcriptome response signatures differentiate ammonium- and nitrate-supplied plants. *Plant Cell Environ* **33**, 1486-1501 (2010).
5. R. Wang *et al.*, Genomic analysis of the nitrate response using a nitrate reductase-null mutant of Arabidopsis. *Plant Physiol* **136**, 2512-2522 (2004).
6. M. S. Katari *et al.*, VirtualPlant: a software platform to support systems biology research. *Plant Physiol* **152**, 500-515 (2010).
7. M. L. Gifford, A. Dean, R. A. Gutierrez, G. M. Coruzzi, K. D. Birnbaum, Cell-specific nitrogen responses mediate developmental plasticity. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 803-808 (2008).
8. R. C. O'Malley *et al.*, Cistrome and Epicistrome Features Shape the Regulatory DNA Landscape. *Cell* **165**, 1280-1292 (2016).
9. E. Bouguyon *et al.*, Multiple mechanisms of nitrate sensing by Arabidopsis nitrate transceptor NRT1.1. *Nature Plants* **1**, (2015).
10. R. Wang, X. Xing, Y. Wang, A. Tran, N. M. Crawford, A genetic screen for nitrate regulatory mutants captures the nitrate transporter gene NRT1.1. *Plant Physiol* **151**, 472-478 (2009).
11. M. D. Brooks *et al.*, Network Walking charts transcriptional dynamics of nitrogen signaling by integrating validated and predicted genome-wide interactions. *Nat Commun* **10**, 1569 (2019).