Supporting Information:

1. Materials and Methods
2. Supplementary Figures S1-S15
3. Supplementary Tables S1-S4
4. References

1. Materials and Method

Plant materials and growth conditions. All *Arabidopsis* thaliana plants were of Columbia ecotype. *tcp20* (SALK_088460C), *nlp6* (SALK_ 036557) and *nlp7* mutants (SALK_ 26134) were obtained from ABRC. *nlp6tcp20*, *nlp7tcp20* and *nlp6nlp7* double mutants were produced by crossing the two parental lines, selfing the F1 plants, and selecting F2 homozygous progeny homozygous by PCR analysis. Basal medium contained 10 mM KH_2PO_4/K_2HPO_4 (pH 5.8), 2 mM $MgSO_4$, 1 mM $CaCl_2$, 0.125 mM NaFeEDTA, 0.5% (wt/vol) sucrose, 0.125 mM H_3BO_3, 0.03 mM $MnSO_4$, 2.5 mM $ZnSO_4$, 2.5 mM $CuSO_4$, 0.5 mM Na_2MoO_4, and 0.5% agarose. This basal medium was complemented with KNO_3/NH_4NO_3/ammonium succinate as the sole nitrogen source at the concentration indicated for each individual experiment. After storing for 2 d at 4 °C in the dark, plates were vertically positioned in a growth chamber at 23 °C for 6-7d with a 24-h light and light intensity of 140 μmol $m^{-2}s^{-1}$ unless otherwise descriptions. For N starvation treatment, 5 mM KCl was added to N-free medium plates to balance ion strength. *N. benthamiana* seeds were first germinated in soil. The two- to three-leaf stage seedlings were then washed with water to get rid of soil, and transferred to perlite. Hydroponic solution used for *N. bethamiana* growth contains the same components as the basal medium except for the addition of sucrose and agarose.

Plasmid constructions. Gibson DNA assembly method was employed to all cloning steps (46) unless otherwise descriptions. The DNA binding regions of TCP20 (TCP20-DB), NLP6 (NLP6-DB) and NLP7 (NLP7-DB) were amplified with the primers listed in Table S2 and then cloned to pGEX-2TK. For yeast-two-hybrid, the full length of *TCP20* was cloned into the sites EcoR I and Xho I of pB42AD vector. The EcoR I end was filled with dNTP and became blend end. The PCR products of full length TCP20 were digested by Xho I and then treated with T4

polynucleotide kinase for 5' end phosphorylation before ligation. The full length of NLP6/7 was cloned into pGilda vectors. For TCP20-YFP, NLP6-YFP and NLP7-YFP constructs, the full length of TCP20, NLP6 and NLP7 were cloned into the NcoI site between 35S promoter and YFP in a pGREEN II0229 vector.

Protein expression and purification. The growth conditions of Escherichia coli cells (strain BL21, Biopioneer) harboring GST expression constructs (pGEX-2T) were described (16, see main text reference). The detailed purification steps were as described (47).

Electrophoretic mobility shift assay (EMSA). Briefly, Each oligonucleotide pair was annealed in buffer containing 10mM Tris, 1 mM EDTA and 50 mM NaCl, pH 8.0 by using 95°C, 5min; 70 cycles 95°C (-1C/cycle), 1 min; 4°C hold in a thermocycler. Then double-stranded small DNAs were purified by 12% non-denature acrylamide gel. The right size bands were cut and crashed in TE (pH8.0) buffer. Gel slurry was pressed through a 0.45μm sterile cellulose acetate filter to remove gel particles. Amicon Ultra-0.5 centrifugal filter devices were used to concentrate DNAs and remove salt. All DNA probes contain four-nucleotide-overhang at 3' end. DNA probes were then labelled by using Biotin 3' end DNA labeling kit (Thermo).

Yeast two-hybrids (Y2H). The LexA inducible system was applied for Y2H. pGilda and pB42AD constructs were cotransformed into EGY48 yeast strain (-Ura). The corresponding empty vector was used as negative controls. Positive colonies were selected on plates (–Ura, -His, -Trp + glucose) and then verified by PCR. Induction of protein-protein interaction was tested by growing the resulting yeast strains on plates in the presence of galactose and raffinose (DB Falcon). X-gal was used for blue and white colorimetric assays on plates.

Bimolecular florescence complementation and transient expression. Briefly, full-length or all of deletion derivatives *TCP20*, *NLP6*, *NLP7* cDNA were directly cloned into pBJ36-SPYNE (YFP N-terminal portion) and pBJ36-SPYCE (YFP C-terminal portion) vector by Gibson DNA assembling. Each cassette was then cut and cloned into the Not I site of pGreenII0179 (SPYCE cassettes) or pGreenII0229 (SPYNE cassettes) (48). All the constructs were transformed into the *Agrobacterium tumefaciens* AGL-0 strain in BiFC experiment. *Agrobacterium* solution was adjusted to OD600 nm = 0.5 and then equally mixed before Agroinfiltration. An *Agrobacterium* strain carrying the viral suppressor p19 (49) and an *Agrobacterium* strain containing mCherry-NLS (50) were always used for in this experiment. Middle leaves of juvenile *N. bethamiana* plants at eight-leaf-stage were used for Agroinfiltration. The detailed procedures were described in (33, see main text reference).

Transgenic lines. Transgenic *Arabidopsis* plants were produced by the floral-dip procedure using 4-week-old plants and *Agrobacterium tumefaciens* cultures containing the appropriate constructs(51). Seeds from treated plants were collected and screened. Transgenic plants carrying both 35S::TCP20-cYFP and 35S::NLP6/7-nYFP were produced by crossing the two T1 parental lines (each contains one BiFC construct). The seedlings that grow on the selective media containing hygromycin and Basta have been used for experiments.

Confocal microscopy. The infiltrated N. bethamiana leaves were imaged using a Zeiss MLS 710 confocal microscope after 3 days Agroinfiltration. YFP signals (Green) and mCherry (Red) signals were captured at the same time from different detection channels. A 514 nm laser line was used to stimulate YFP and mCherry. A 514-546 nm bandpass filter was used to collect YFP signal and a 610-644 nm bandpass filter was used to collect mCherry signal. To visualize root anatomy, primary roots were stained in 10 mg/L propidium iodide (PI) for 4–6 min, rinsed and

mounted in water. A 584-744 nm bandpass filter was used to collect PI signals. Image J was used to process all the images.

Real-time quantitative polymerase chain reaction (qPCR). Total RNA was prepared from total roots using an RNeasy Plant Mini kit (Qiagen) and quantified with a Genesis 6 spectrophotometer (ThermoSpectronic). Template cDNA samples were prepared using the SuperScript First-Strand Synthesis System kit (Invitrogen) for reverse transcription with 0.5 μg of total RNA in a reaction volume of 15 μL. The cDNA synthesis reaction mixture was diluted 10 times before being used for qPCR. Real-time quantitative PCR was performed using a LightCycler system from Roche Diagnostics. Primers for the PCR reactions were designed to have a melting temperature of about 60–65 °C and to give a PCR product between 175 and 250 bp. The primers are listed in Table S3. The gene expression level was normalized by Ubiquitin 5.

2. Supplementary Figures

Fig. S1. Identifications of TCP20 and NLP6&7 binding elements in the 109-bp *NIA1* enhancer fragment.

Fig. S2. TCP20 -NLP6&7 interactions in transgenic *Arabidopsis* roots.

Fig. S3. BiFC analysis of TCP20-TCP20 interactions and empty vectors.

Fig. S4. Protein-protein interaction in NLPs under nitrate-grown condition.

Fig. S5. The subcellular localization of TCP20-YFP, NLP6-YFP and NLP7-YFP chimeric protein fusions in *N. benthamiana* transient expression system (**A-F**) and transgenic *Arabisopsis* (**G-L**).

Fig. S6. The subcellular locations of TCP20 -NLP6&7 interactions under nitrate-grown condition.

Fig. S7. The subcellular locations of TCP20 -NLP6&7 interactions in transgenic *Arabidopsis* roots.

Fig. S8. Protein-protein interaction in NLPs under N starvation.

Fig. S9. Amino acid sequence alignment of NLP6&7 PB1 domains and the C-terminal domains of TCP20 from different species by using Clustal Omega.

Fig. S10. A PB1 domain of NLPs is important for their protein-protein interaction.

Fig. S11. BiFC assay of interactions between NLP6&7 and deleted derivatives of TCP20.

Fig. S12. NLP6 and NLP7 are partially redundant activators of nitrate transport, assimilation and signaling genes under nitrate growth condition (5 mM KNO_3).

Fig. S13. Phenotype of *nlp6nlp7* double mutants under different nitrogen growth conditions.

Fig. S14. Proximal *CYCB1;1* promoter region.

Fig. S15. Root phenotype under 5 mM nitrate (A) or N starvation (B), primary root length (C) and root meristem length (C) of WT, single mutants and double mutants under N starvation treatment.

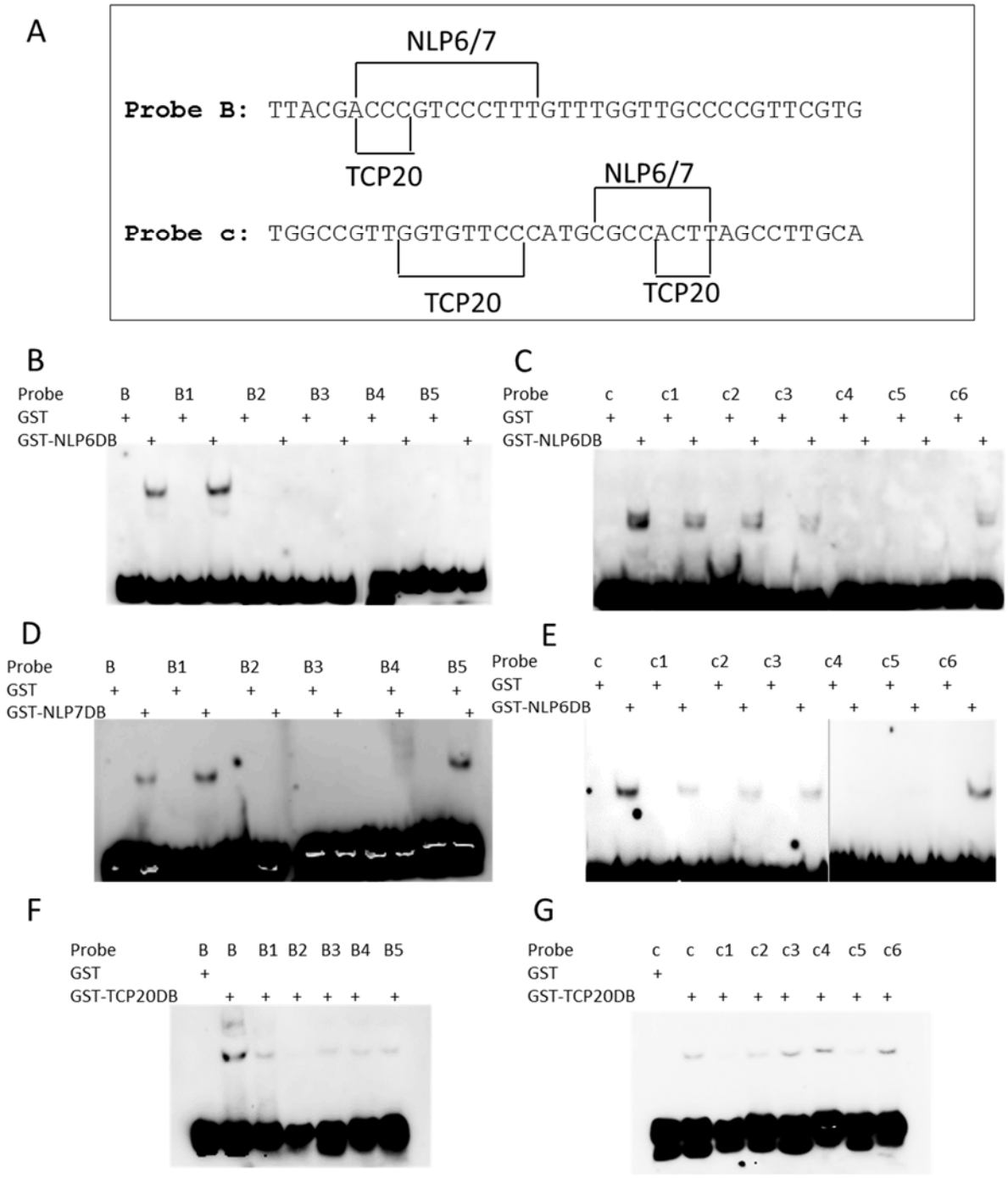

Figure S1. Identifications of TCP20 and NLP6&7 binding elements in the 109-bp *NIA1* enhancer fragment. **a**. Diagram of the TCP20 and NLP6&7 binding sites in probes B and C, respectively. **B-G**, EMSA experiments for testing NLP6DB (**B**, **C**), NLP7DB (**D**, **E**) and TCP20DB (**F**, **G**). Mutated probes (B1-B5 and c1-c6) have been used in EMSA to determine DNA *cis*-elements. All the probes are listed in Table S1.

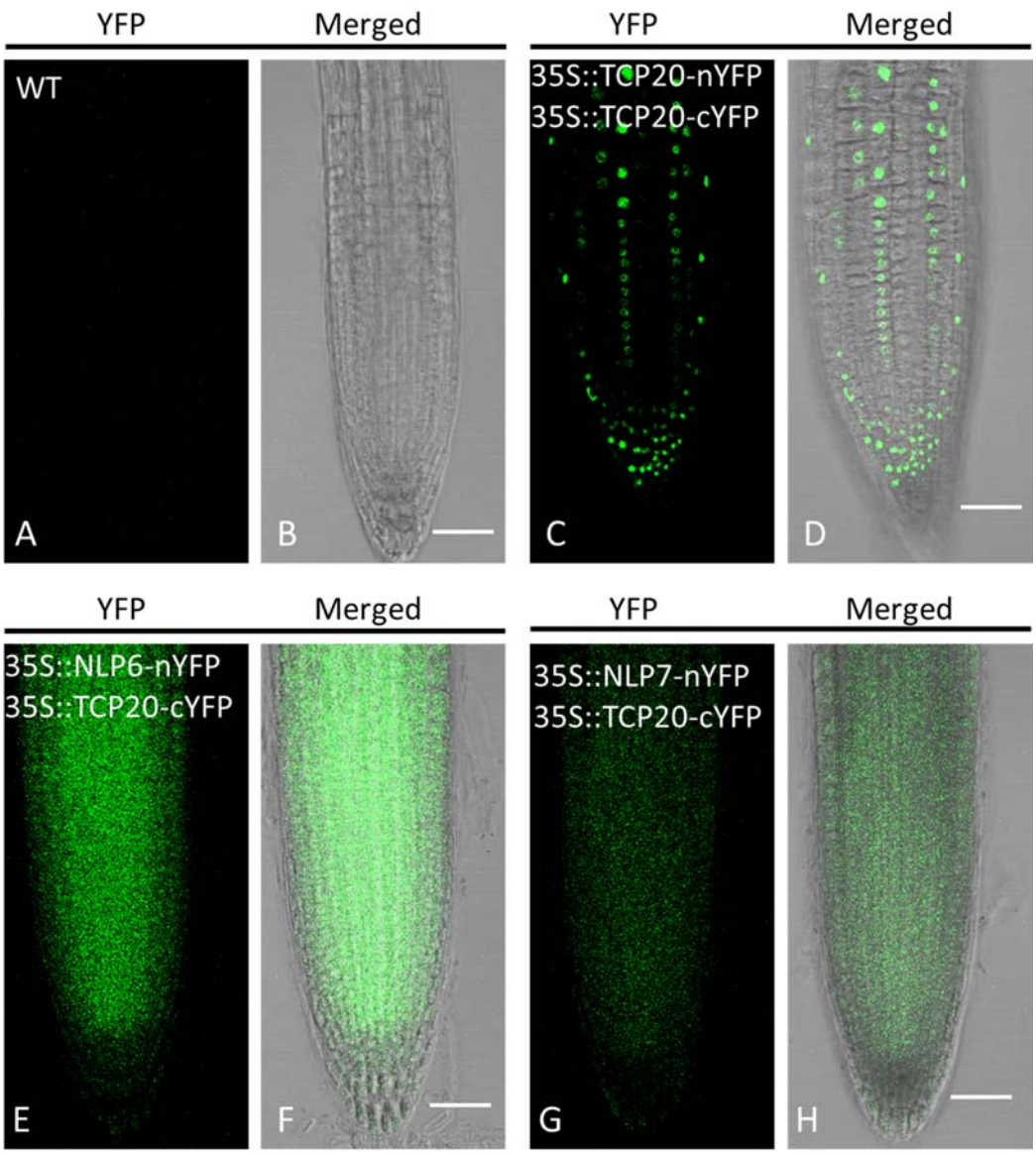

Figure S2. TCP20 -NLP6&7 interactions in transgenic *Arabidopsis* roots. BiFC constructs (NLP6-nYFP, NLP7-nYFP, TCP20-nYFP and TCP20-cYFP) were transformed into *Arabidopsis*. T1 plants were then crossed to obtain the progenies carrying both nYFP and cYFP derivatives. Scale bar: 50 μm (**A-H**).

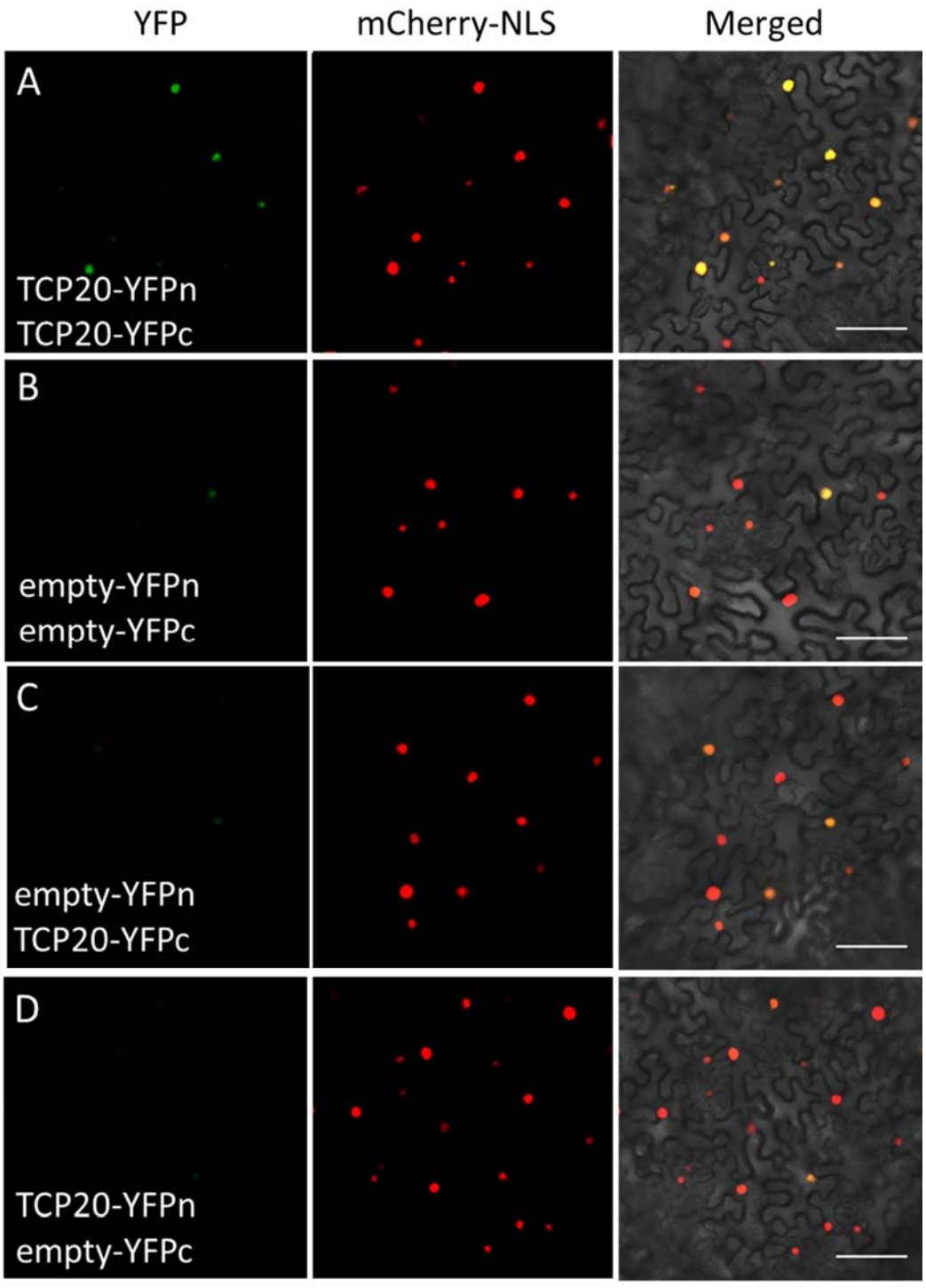

Figure S3. BiFC analysis of TCP20-TCP20 interactions and empty vectors. BiFC was conducted in leaves of *N. benthamiana* grown on 5 mM KNO_3 as N source. A mCherry-NLS (Nuclear Localization Signal) version was used as a nuclear marker. Scale bar: 100 μm.

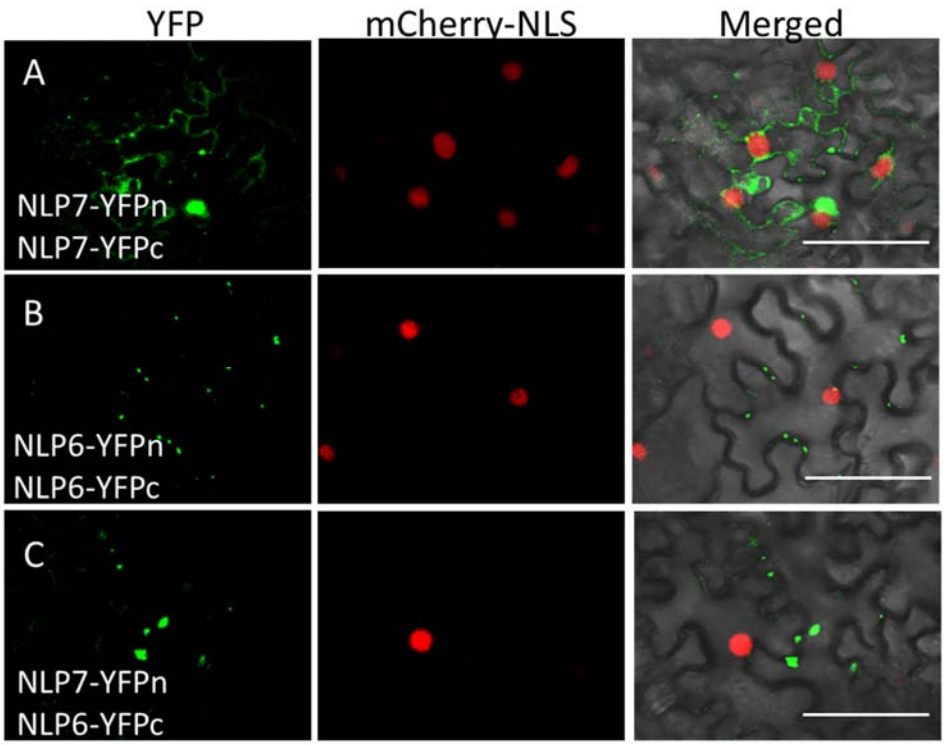

Figure S4. Protein-protein interaction in NLPs under nitrate-grown condition. BiFC was conducted in leaves of *N. benthamiana* grown on 5 mM KNO_3 as N source. mCherry-NLS was used as a nuclear marker. Scale bar: 100 μm.

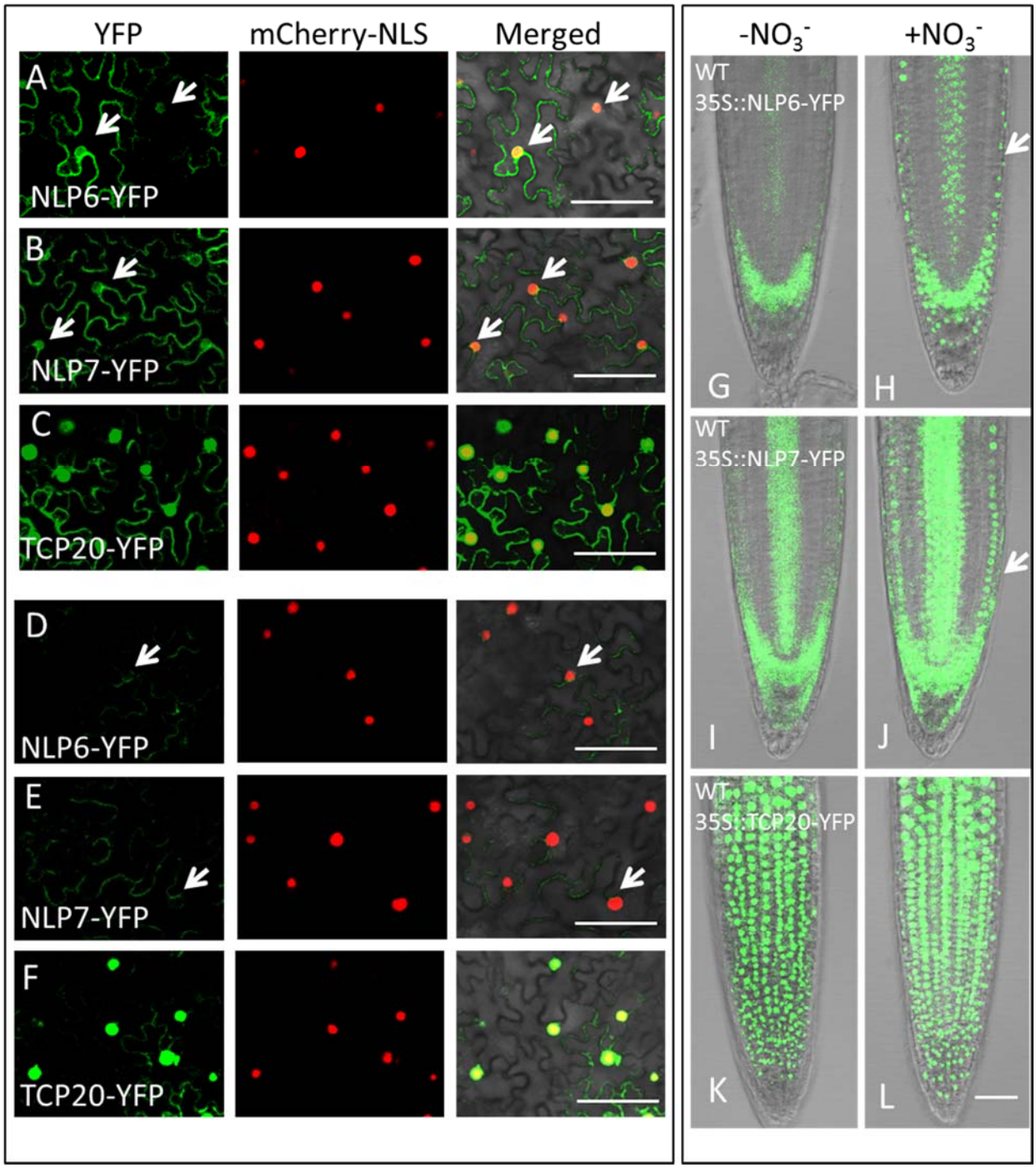

Figure S5. The subcellular localization of TCP20-YFP, NLP6-YFP and NLP7-YFP chimeric protein fusions in *N. benthamiana* transient expression system (**A-F**) and transgenic *Arabidopsis* (**G-L**). *N. benthamiana* was grown on 5 mM KNO_3 as sole N source (**A-C**) or under N starvation condition (**D-F**). Transgenic *Arabidopsis* seedlings were treated with N free medium (**G**, **I** and **K**) or nitrate medium containing 5 mM KNO_3 (**H**, **J** and **L**).White arrows indicate nucleus. Scale bar: 100 μm (**A-F**), 50 μm (**G-L**).

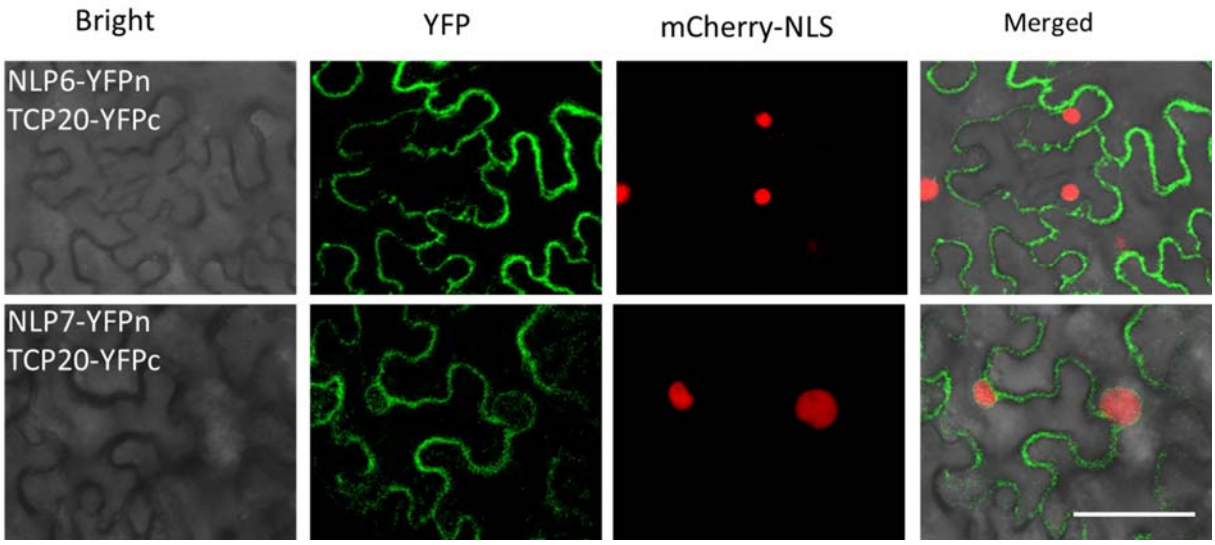

Figure S6. The subcellular locations of TCP20 -NLP6&7 interactions under nitrate-grown condition. *N. benthamiana* was grown on 5 mM KNO_3 as sole N source. Scale bar: 100 μm.

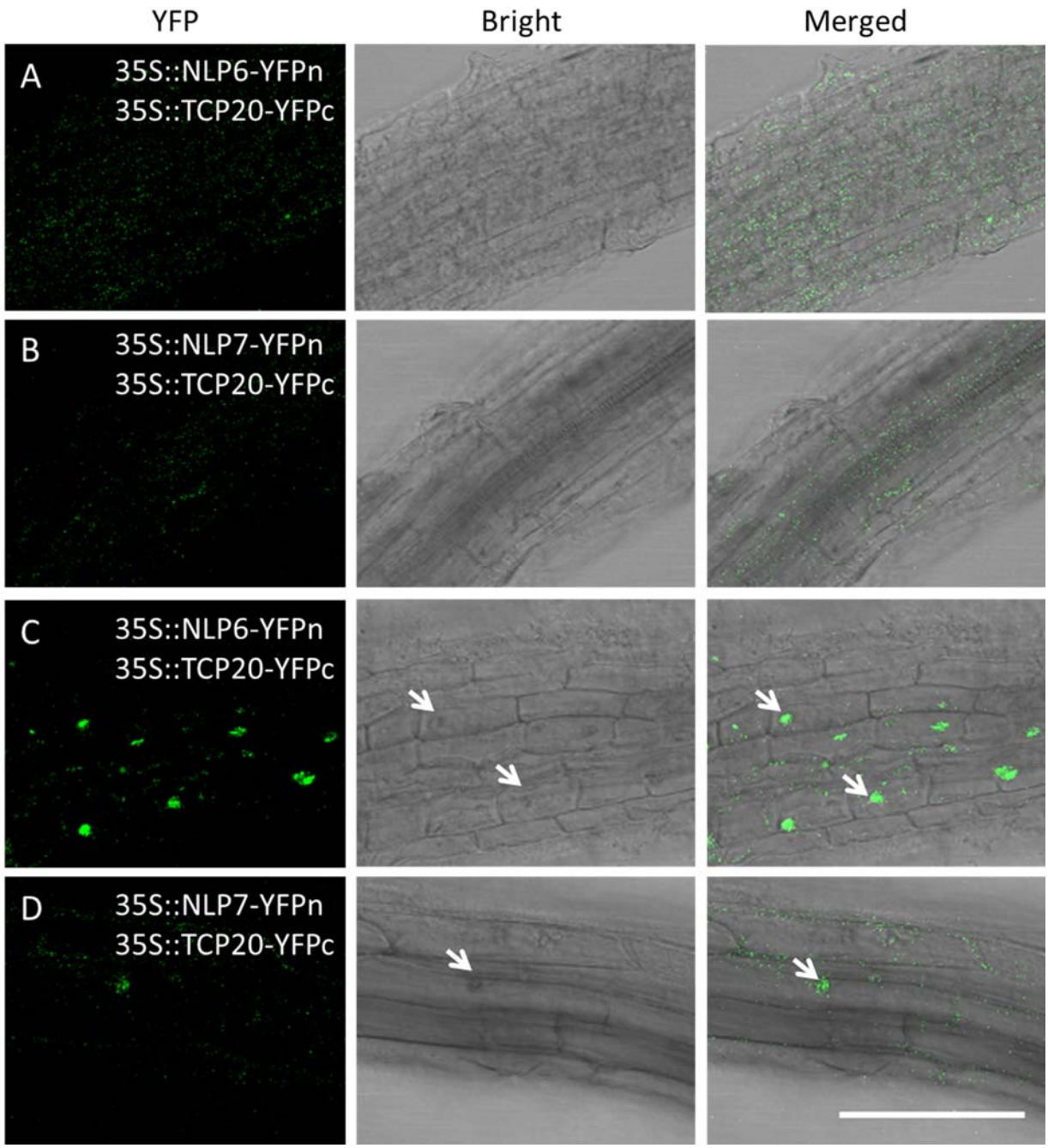

Figure S7. The subcellular locations of TCP20 -NLP6&7 interactions in transgenic *Arabidopsis* roots. Transgenic *Arabidopsis* seedlings grown on 5 mM KNO_3 as sole N source (**A-B**) and then transferred to N free plates for 2 days (**C-D**). White arrows indicate nucleus. Scale bar: 100 μm.

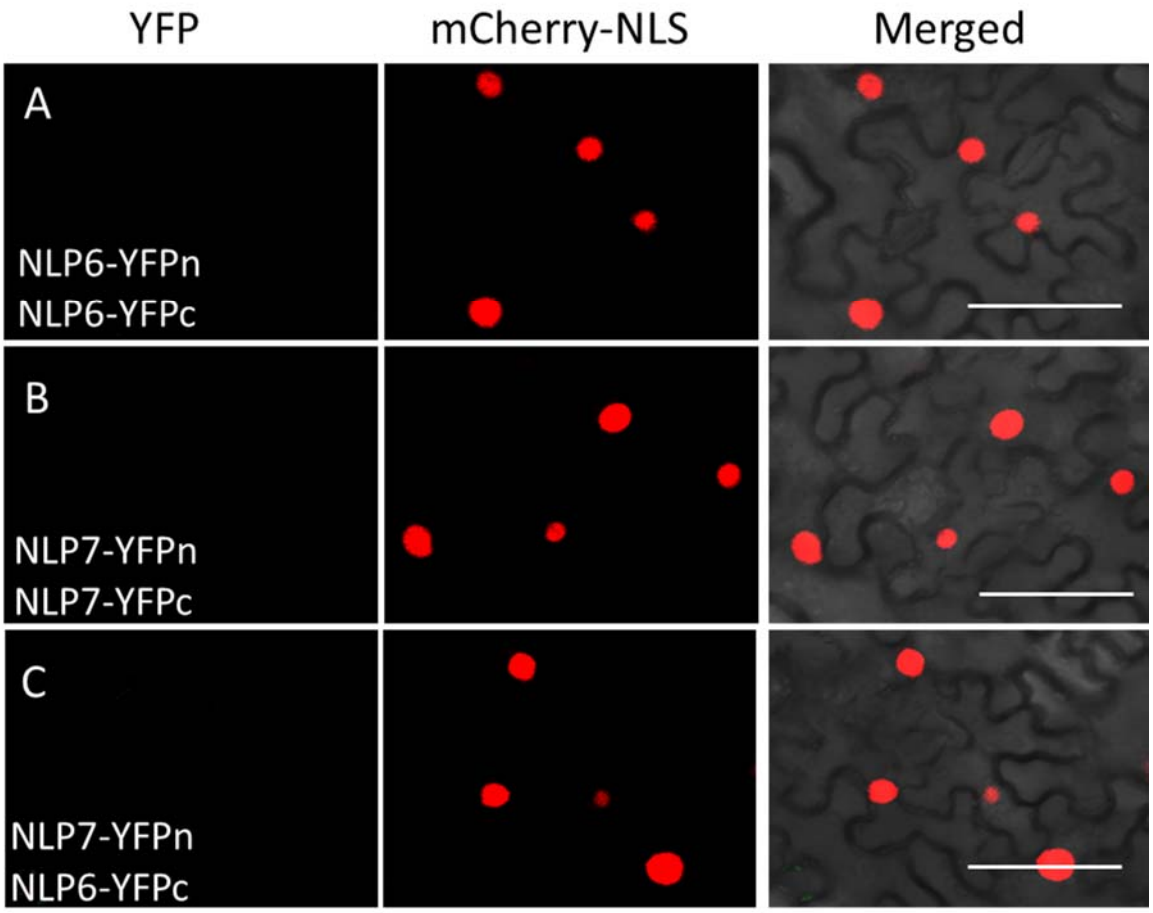

Figure S8. Protein-protein interaction in NLPs under N starvation. *N. benthamiana* was grown on 5 mM KNO_3 as sole N source and then received N starvation treatment. Scale bar:100 μm.

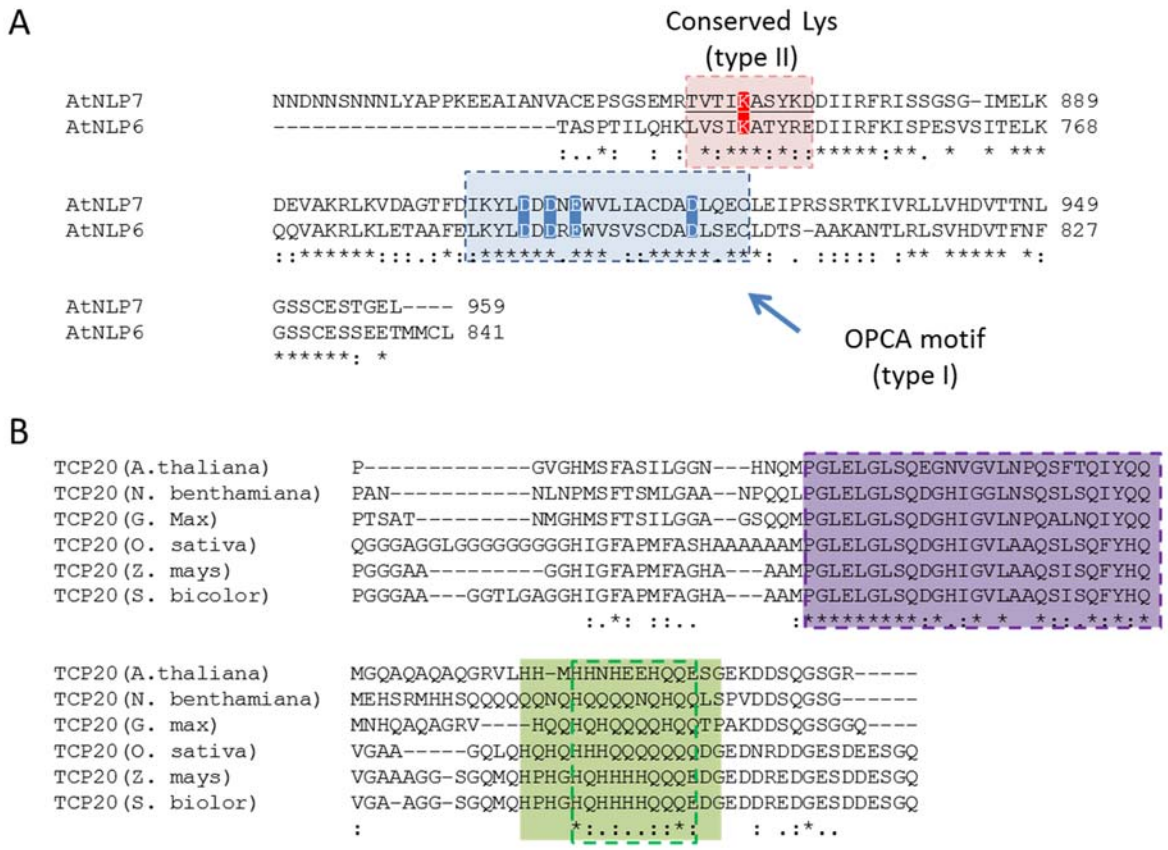

Figure S9. Amino acid sequence alignment of NLP6&7 PB1 domains (**A**) and the C-terminal domains of TCP20 from different species (**B**) by using Clustal Omega. **A**, NLP6&7 contains type I/II PB1 domain. Conserved lysine residue (K) is indicated in red; conserved acidic residues within the OPCA motif are highlighted in blue. **B**, Two conserved regions of TCP20 are indicated in purple and green. HQ-rich domains in TCP20 C-terminus (shown in green) have been identified by online source (ExPASy-MyHits, http://myhits.isb-sib.ch/). Asterisks indicate amino acids are conserved. Dots indicate amino acids are similar.

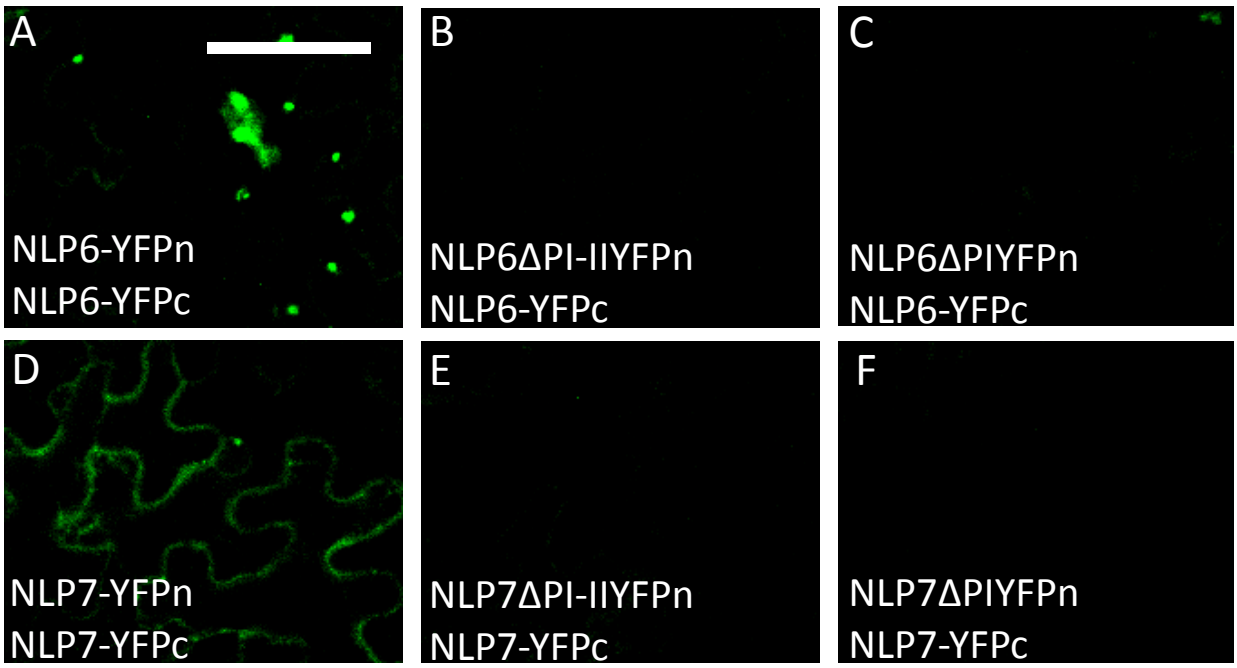

Figure S10. A PB1 domain of NLPs is important for their protein-protein interaction. BiFC assay for testing NLP6&7 deleted derivatives. *N. benthamiana* were grown on 5 mM KNO_3 as sole N source. Scale bar: 100 μm.

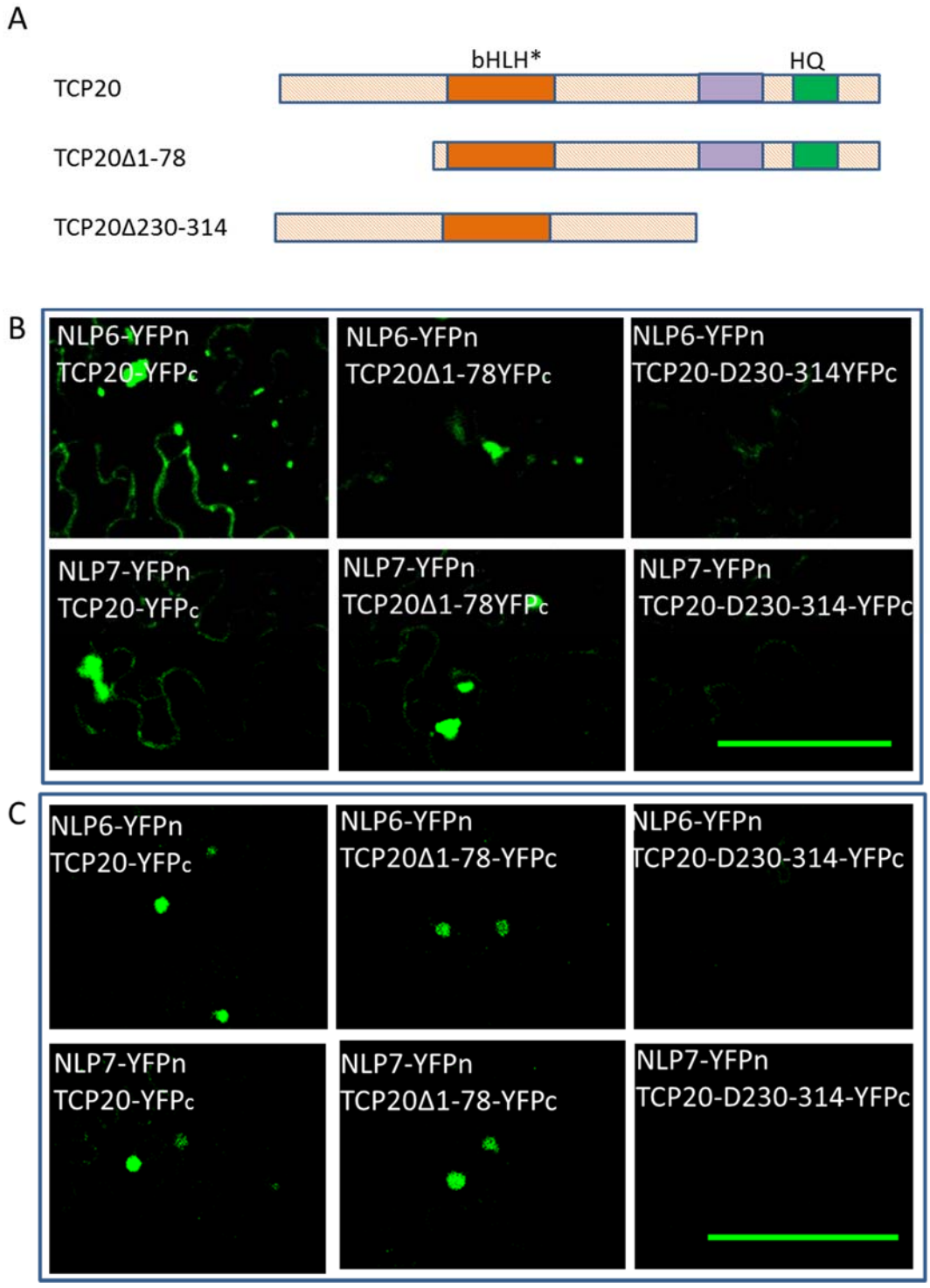

Figure S11. BiFC assay of interactions between NLP6&7 and deleted derivatives of TCP20. **A**. Schematic diagram of the TCP20 deletion derivatives. Orange and green boxes present bHLH* and histidine glutamine rich domain (HQ) in TCP20. Purple box presents a conserved region adjacent to HQ module. **B**, BiFC was conducted in *N. benthamiana* grown on 5mm KNO_3 as sole N source. **C**, The experiment was conducted in N starved plants. Scale bar: 100 μm.

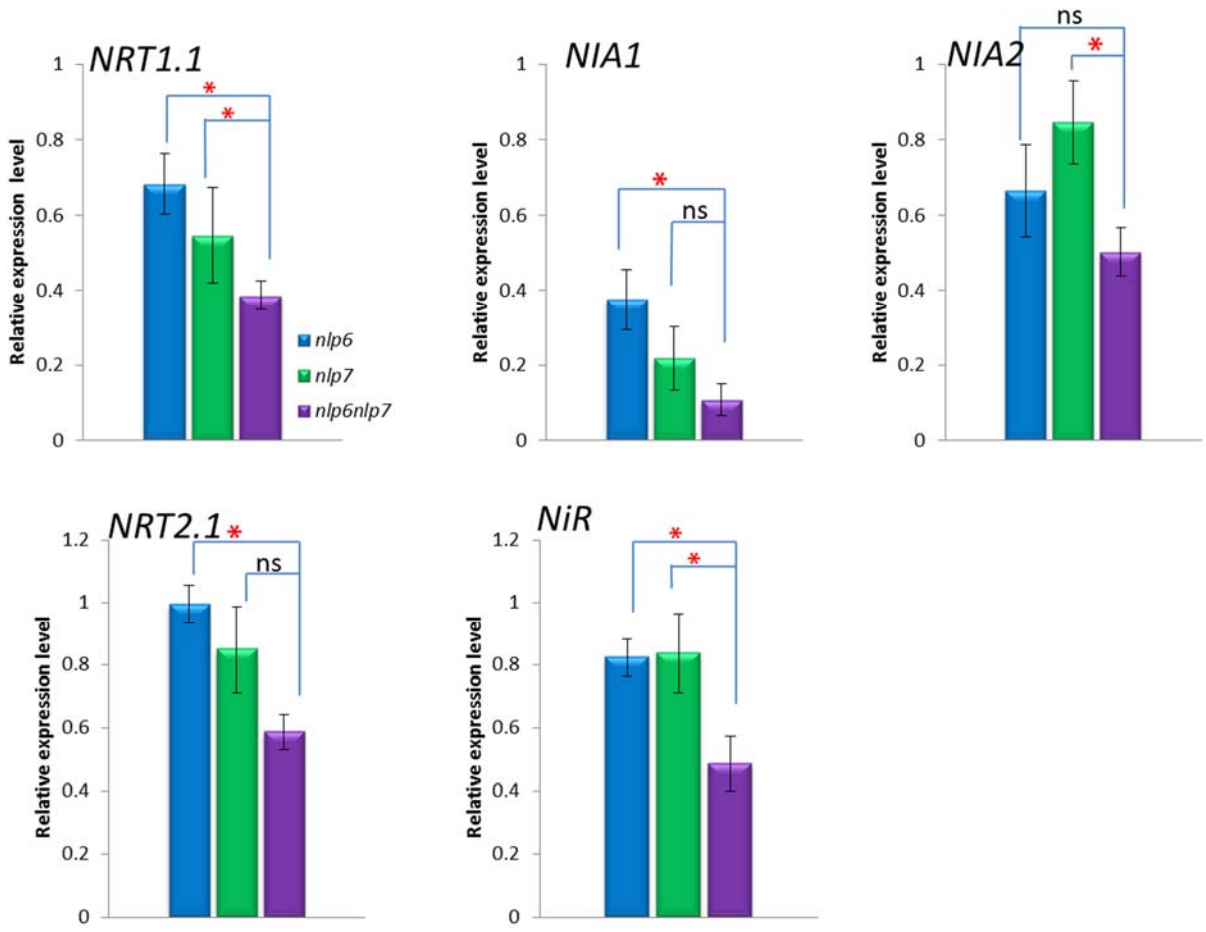

Figure S12. NLP6 and NLP7 are partially redundant activators of nitrate transport, assimilation and signaling genes under nitrate growth condition (5 mM KNO_3). RT-PCR quantification of target gene expression levels in whole roots of WT and mutant lines was performed. Error bars show mean+SEM (n = 3 biological replicates). Red asterisks represent significant statistical difference between two indicated lines. *$p<0.05$ (t-test).

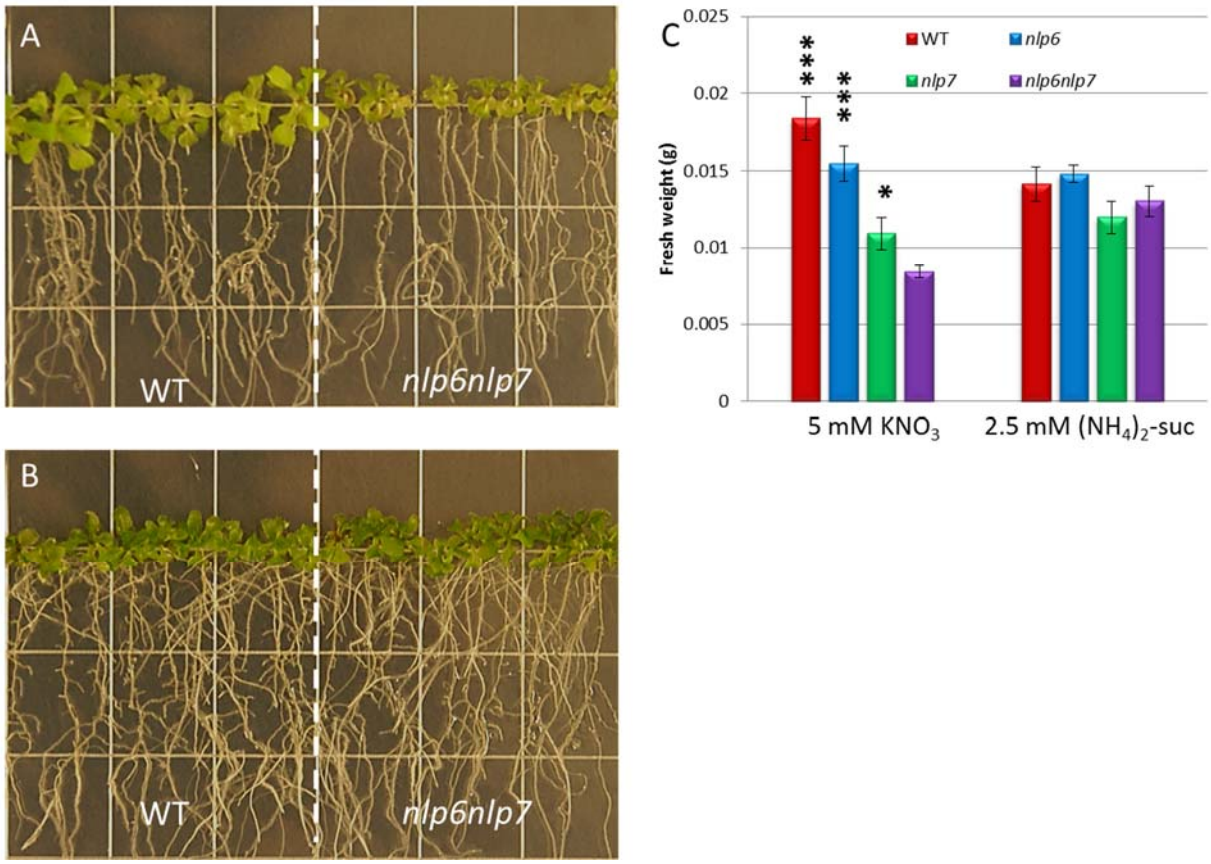

Figure S13. Phenotypes of *nlp6nlp7* double mutants under different nitrogen growth conditions. **A**, 5 mM nitrate grown. **B**, 2.5 mM $(NH_4)_2$-succinate. **C**. fresh weight of WT, *nlp6*, *nlp7* and *nlp6nlp7* mutants. Error bars show SEM (n =9). Black asterisks represent significant statistical difference compared to *nlp6nlp7* mutant. *p<0.05, **P<0.01, ***P<0.001 (t-test).

Figure S14. Proximal *CYCB1;1* promoter region. Putative NLP6&7-binding sites are indicated with green boxes. Red underlines and yellow box are indicated TCP20 and auxin response factor (ARF)-binding sites respectively, which have been proposed in Li, et al [30]. Blue arrow represents the transcription start site. The start codon is highlighted with a blue box.

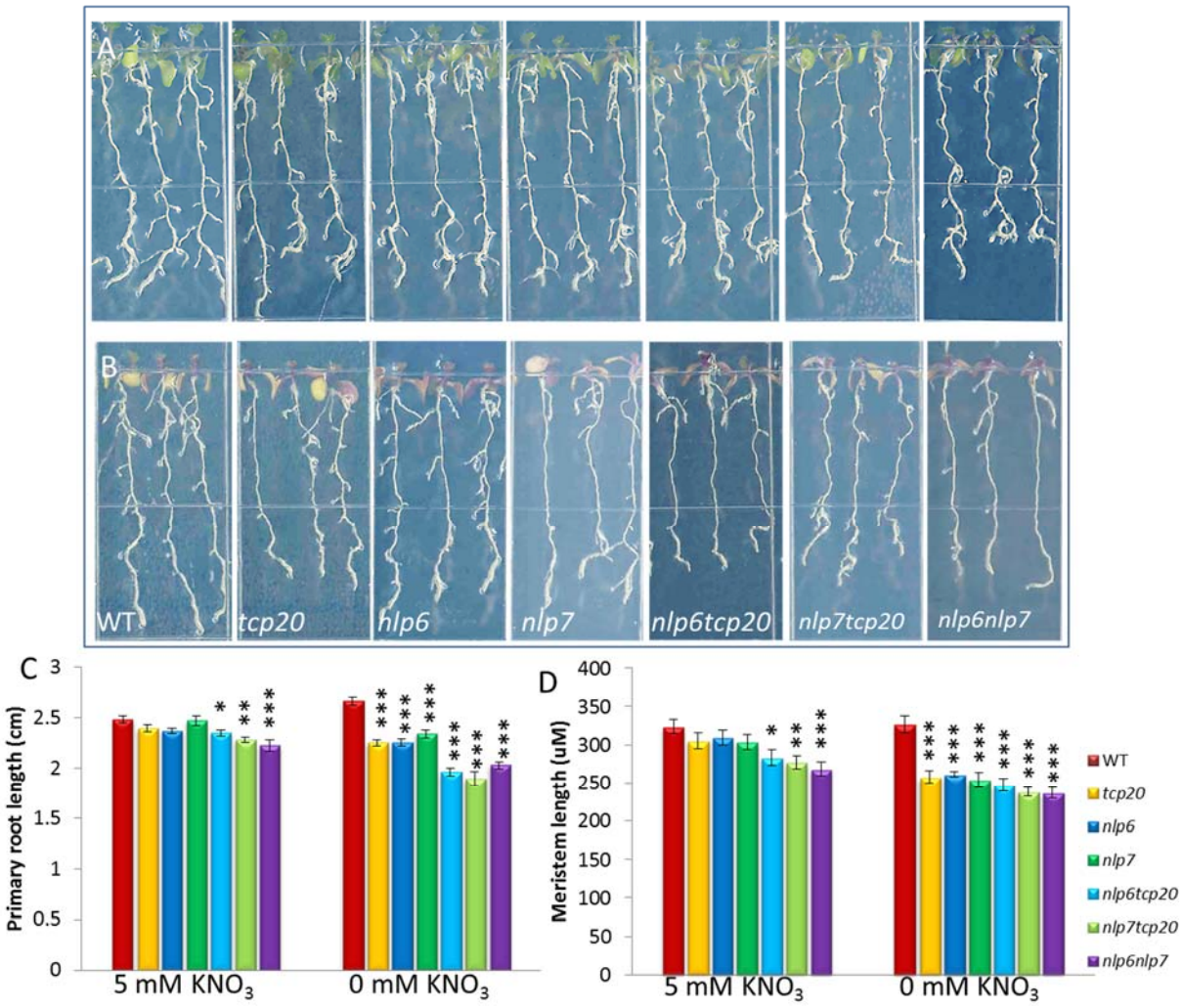

Figure S15. Root phenotype under 5 mM nitrate (**A**) or N starvation (**B**), primary root length (**C**) and root meristem length (**D**) of WT, single mutants and double mutants under N starvation treatment. For root phenotype and primary root length, *Arabidopsis* seedlings were grown on 5 mM KNO_3 plates for 6-7days and then were transferred to new 5 mM KNO_3 plates or N free plates for three days. Error bars show SEM (n =18). For measuring meristem length, Seedlings were grown on 5 mM KNO_3 medium for 3 d and then were transferred to N free medium for 3d with a 8-h light. Error bars show SEM (n =10). One-way ANOVA was performed and followed by t-test to calculate the p-value (using WT as control). Black asterisks represent statistic difference between WT and mutants under same nitrate condition. *p<0.05, **P<0.01, ***P<0.001.

3. Supplementary Tables.

Table S1. Probes used in this work. All the probes are listed as below. Orange letters represent mutated nucleotides in the probes.

NIA-109 bp:
TCGAAAATTCAAACAAGTGACAAAAATAATATTACGACCCGTCCCTTTGTTTGGTTGC
CCCGTTCGTGTATTTGCTTGGCCGTTGGTGTTCCCATGCGCCACTTAGCCT

A: TCGAAAATTCAAACAAGTGACAAAAATAATATTA
B: TTACGACCCGTCCCTTTGTTTGGTTGCCCCGTTCGTG
C: GTGTATTTGCTTGGCCGTTGGTGTTCCCATGCGCCACTTAGCCT
D: CAAGTGACAAAAATAATATTACGACCCGTCCCTTTG
E: GTTGCCCCGTTCGTGTATTTGCTTGGCCGTTGGTG

B1: TCTGTACCCGTCCCTTTGTTTGGTTGCCCCGTTCGTG
B2: TTACGCGAAGTCCCTTTGTTTGGTTGCCCCGTTCGTG
B3: TTACGACCCACAACTTTGTTTGGTTGCCCCGTTCGTG
B4: TTACGACCCGTCCTCCCGTTTGGTTGCCCCGTTCGTG
B5: TTACGACCCGTCCCTTTACACGGTTGCCCCGTTCGTG
C: TGGCCGTTGGTGTTCCCATGCGCCACTTAGCCT
C1: TGGCCGTTATCATTCCCATGCGCCACTTAGCCT
C2: TGGCCGTTGGTGGCATCATGCGCCACTTAGCCT
C3: TGGCCGTTGGTGTTCCTGGACGCCACTTAGCCT
C4: TGGCCGTTGGTGTTCCCATGTAATACTTAGCCT
C5: TGGCCGTTGGTGTTCCCATGCGCCGACCAGCCT
C6: TGGCCGTTGGTGTTCCCATGCGCCACTTGTAAT

Table S2. Primers used in cloning.

TCP20DB-F: GAGGATCC-TCTAACAAAGACAGACACACTAAAG
TCP20DB-R: TGAACCAGTAGCTGCAATAATC
NLP6DB-F: GAGGATCCGTGAAAAAATCAGAAAGGAAG
NLP6DB-R: AGCACCTTGAACCGAGTC
NLP7DB-F: AAGAAGAAAACAGAGAAAAAG
NLP7DB-R: GCGGATCCAGTACCTTGAACAGATTCG
TCP20pB42AD-F: CATGGATCCCAAGAACCTAAATCG
TCP20pB42AD-R: CTCTCGAGTTAACGACCTGAGCCTTGAG
NLP6pGilda-F: AACGGCGACTGGCTGGAATTCATGGAACTTGACGACTTG
NLP6pGilda-R: TTGGCTGCAGGTCGACTCGAGTCACAAGCACATCATAGTTTC
NLP7pGilda-F: AACGGCGACTGGCTGGAATTCATGTGCGAGCCCGATGATAATTC
NLP7pGilda-R: TTGGCTGCAGGTCGACTCGAGTCACAATTCTCCAGTGCTCTCG
TCP20-NcoI-F: CACCATTTACGAACGATAGCATGGATCCCAAGAACCTAAATCG
TCP20-NcoI-R:
AGCTCCTCGCCCTTGCTCACCATGGCAGCACGACCTGAGCCTTGAGAATC
NLP6-NcoI-F: CACCATTTACGAACGATAGCATGGAACTTGACGACTTG
NLP6-NcoI-R: AGCTCCTCGCCCTTGCTCACCATGGCAGCCAAGCACATCATAGTTTCC
NLP6-Nco-F: CACCATTTACGAACGATAGCATGTGCGAGCCCGATGATAATTCC
NLP6-Nco-R: AGCTCCTCGCCCTTGCTCACCATGGCAGCCAATTCTCCAGTGCTCTCG
TCP20-BIFCF2: CAATTACTATTTACAATTGTCGACATGGATCCCAAGAACCTAAATCG
TCP20-BIFCR2: GATTTCGAACCCGGGGTACCACGACCTGAGCCTTGAG
TCP20Δ72 BIFC2:
CAATTACTATTTACAATTGTCGACATGAAAGACAGACACACTAAAG
TCP20Δ230R: GATTTCGAACCCGGGGTACCAAAATCAAAACCAGGAAACC
TCP20Δ270R: GATTTCGAACCCGGGGTACCAAAAGACTGAGGATTCAAAAC
TCP20ΔHLH-R: CAATAATCGAAAGAGCAGGCATCCGAATTCGTCGACCTC
TCP20ΔHLH-F: GCCTGCTCTTTCGATTATTGCAGCTACTGGTTCAGG
NLP6-BIFCF2: CAATTACTATTTACAATTGTCGACATGGAACTTGACGACTTGG
NLP6-BIFCR2: GATTTCGAACCCGGGGTACCCAAGCACATCATAGTTTCC
NLP6R ΔPI-II-R: GATTTCGAACCCGGGGTACCGAGCTTATGTTGGAGAATTG
NLP6 ΔPI-R: GATTTCGAACCCGGGGTACCCCTTTTGGCCACTTGCTG
NLP7-BIFCF2: CAATTACTATTTACAATTGTCGACATGTGCGAGCCCGATGATAATTCC
NLP7-BIFCR2: GATTTCGAACCCGGGGTACCCAATTCTCCAGTGCTCTCG
NLP7ΔPI-II-R: GATTTCGAACCCGGGGTACCCGTTCTCATTTCTGAGCCTGATGG
NLP7 ΔPI-R: GATTTCGAACCCGGGGTACCCCTCTTAGCCACTTCATCC

Table S3. Primers used in qPCR.

Gene	Forward primer	Reverse primer
NIA1	GGTGCTGGTGTTTCTGGTCACT	GGGTCTGGTCGGGTGTTC
NRT1.1	AAAGCTGCCACACACTGAAC	ATTGTGCGACTGATAATGTCGT
NRT2.1	CCACAGATCCAGTGAAAGG	CATTGTTGGGTGTGTTCTC
NiR	CCGGTAGCCAGTTCTGCG	CCTATTCGTCCCCCGACGT
NIA2	CGAGACACACAACAGCAACGC	GCAACGGGACAGGGGTGA
CYCB1;1	CCAGCACTCTCAAGCATCAC	GGATCAAAGCCACAGCGAAG

Table S4.

(A) Two-way ANOVA analysis of the relative mRNA levels of key genes of nitrate assimilation & signaling and of cell cycle gene, *CYCB1;1* in response to nitrate status.

(B) Two-way ANOVA analysis of primary root meristem cell number, primary root meristem length, and primary root length.

Note: Two independent variables in our experiments are shown as "Sample (Nitrogen)", which means N conditions, i.e. continuous nitrate condition versus N starvation, and as "Columns (Gtype)", which means the genotypes i.e. WT, *tcp20*, *nlp6*, *nlp7, nlp6tcp20, nlp7tcp20* and *nlp6nlp7*.

A

Two-way ANOVA: Relative mRNA level of *NRT1.1*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	0.62	1	0.62	31.93	***4.7E-6	4.20
Columns (Gtype)	0.95	6	0.16	8.23	***3.5E-5	2.45
Interaction (NitrogenxGtype)	0.36	6	0.06	3.14	*0.018	2.45
Within	0.54	28	0.02			
Total	2.48	41				

Two-way ANOVA: Relative mRNA level of *NIA1*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	10.79	1	10.79	45.60	***2.5E-7	4.20
Columns (Gtype)	23.50	6	3.92	16.56	***4.7E-8	2.45
Interaction (NitrogenxGtype)	3.53	6	0.59	2.48	*0.047	2.45
Within	6.62	28	0.24			
Total	44.44	41				

Two-way ANOVA: Relative mRNA level of *NIA2*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	53.24	1	53.24	30.98	***5.9E-6	4.20
Columns (Gtype)	57.82	6	9.64	5.61	***0.001	2.45
Interaction (NitrogenxGtype)	58.29	6	9.71	5.65	***0.001	2.45
Within	48.11	28	1.72			
Total	217.45	41				

Two-way ANOVA: Relative mRNA level of *NRT2.1*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	14.96	1	14.96	0.71	0.408	4.20
Columns (Gtype)	142.56	6	23.76	1.12	0.376	2.45
Interaction (NitrogenxGtype)	119.36	6	19.89	0.94	0.484	2.45
Within	593.77	28	21.21			
Total	870.64	41				

Two-way ANOVA: Relative mRNA level of *NiR*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	76.31	1	76.31	42.90	***4.2E-7	4.20
Columns (Gtype)	16.00	6	2.67	1.50	0.215	2.45
Interaction (NitrogenxGtype)	4.77	6	0.80	0.45	0.841	2.45
Within	49.80	28	1.78			
Total	146.89	41				

Two-way ANOVA: Relative mRNA level of *CYCB1;1*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	0.27	1	0.27	58.09	***2.7E-8	4.20
Columns (Gtype)	0.07	6	0.01	2.47	*0.048	2.45
Interaction (NitrogenxGtype)	0.07	6	0.01	2.45	*0.050	2.45
Within	0.13	28	0.00			
Total	0.54	41				

B

Two-way ANOVA: Primary root meristem cell number

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	651.46	1	651.46	57.75	***6E-12	3.92
Columns (Gtype)	1485.99	6	247.66	21.95	***2E-17	2.17
Interaction (NitrogenxGtype)	149.04	6	24.84	2.20	*0.047	2.17
Within	1421.40	126	11.28			
Total	3707.89	139				

Two-way ANOVA: Primary root meristem length

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	44077.23	1	44077.23	55.86	***1E-11	3.92
Columns (Gtype)	70815.24	6	11802.54	14.96	***7E-13	2.17
Interaction (NitrogenxGtype)	10273.90	6	1712.32	2.17	*0.050	2.17
Within	99429.76	126	789.13			
Total	224596.13	139				

Two-way ANOVA: Primary root length

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample (Nitrogen)	1.77	1	1.77	58.10	***6E-13	3.88
Columns (Gtype)	6.54	6	1.09	35.77	***1E-30	2.14
Interaction (NitrogenxGtype)	1.98	6	0.33	10.81	***1E-10	2.14
Within	7.26	238	0.03			
Total	17.55	251				

4. References.

46. Gibson DG (2011) Enzymatic assembly of overlapping DNA fragments. *Methods Enzymol* 498: 349-361.

47. Mack AM, Crawford NM (2001) The Arabidopsis TAG1 transposase has an N-terminal zinc finger DNA binding domain that recognizes distinct subterminal motifs. *Plant Cell* 13(10):2319–2331.

48. Ripoll JJ, Bailey LJ, Mai QA, Wu SL, Hon CT, Chapman EJ, Ditta GS, Estelle M, and Yanofsky MF (2015) microRNA regulation of fruit growth. *Nature Plants* 1:15036

49. Lincoln C, Long J, Yamaguchi J, Serikawa K, & Hake S (1994) A knotted1-like homeobox gene in Arabidopsis is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants. *Plant Cell* 6 (12): 1859-1876.

50. Wang R, Zhang Y, Kieffer M, Yu H, Kepinski S & Estelle M (2016) HSP90 regulates temperature-dependent seedling growth in Arabidopsis by stabilizing the auxin co-receptor F-box protein TIR1. *Nat Commun* 7:10269.

51. Bechtold N, Ellis J, Pelletier G (1993) In planta Agrobacterium-mediated gene transfer by infiltration of adult Arabidopsis thaliana plants. *C R Acad Sci Paris Life Sci* 316: 1194–119.