

Supplemental materials

Figure S1. a, T-DNA insertion sites of two mutants (*nuc1-1* and *nuc1-3*) [1]. b, Growth phenotypes of 15-day-old seedlings grown on normal 1/2 strength MS medium ($n = 3$). c, Side view of 32-day-old seedlings grown in soil.

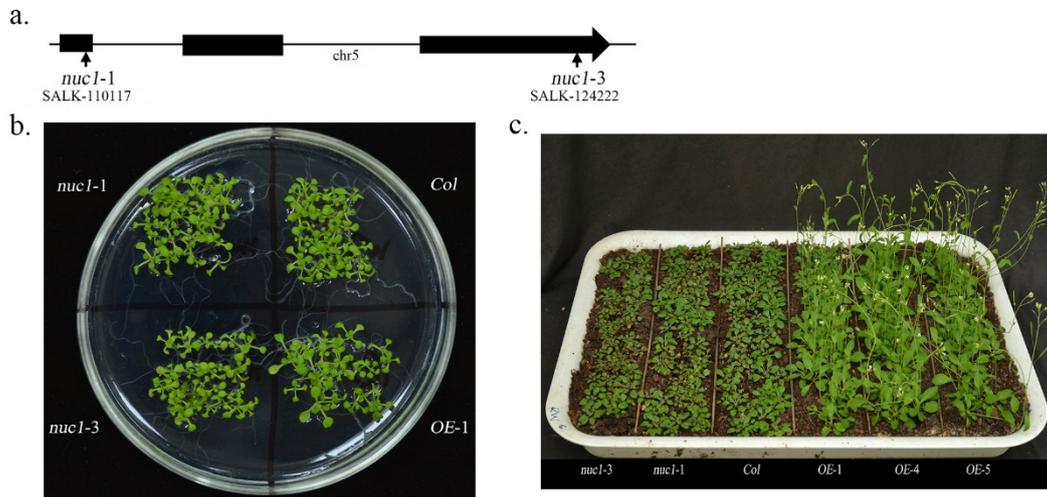


Figure S2. The expression levels of 6 genes that regulate nitrogen metabolism and nitrate response. Four-day-old seedlings grown on normal MS medium were transplanted to MS medium with (CON) or without N (-N) conditions for an additional 6 days. Then, the roots of the lines were harvested for qRT-PCR. Gene expression in *Col* under CON conditions was set to 1.0. The Arabidopsis tubulin gene was used as an external reference. Values are the mean \pm SDs ($n = 3$)

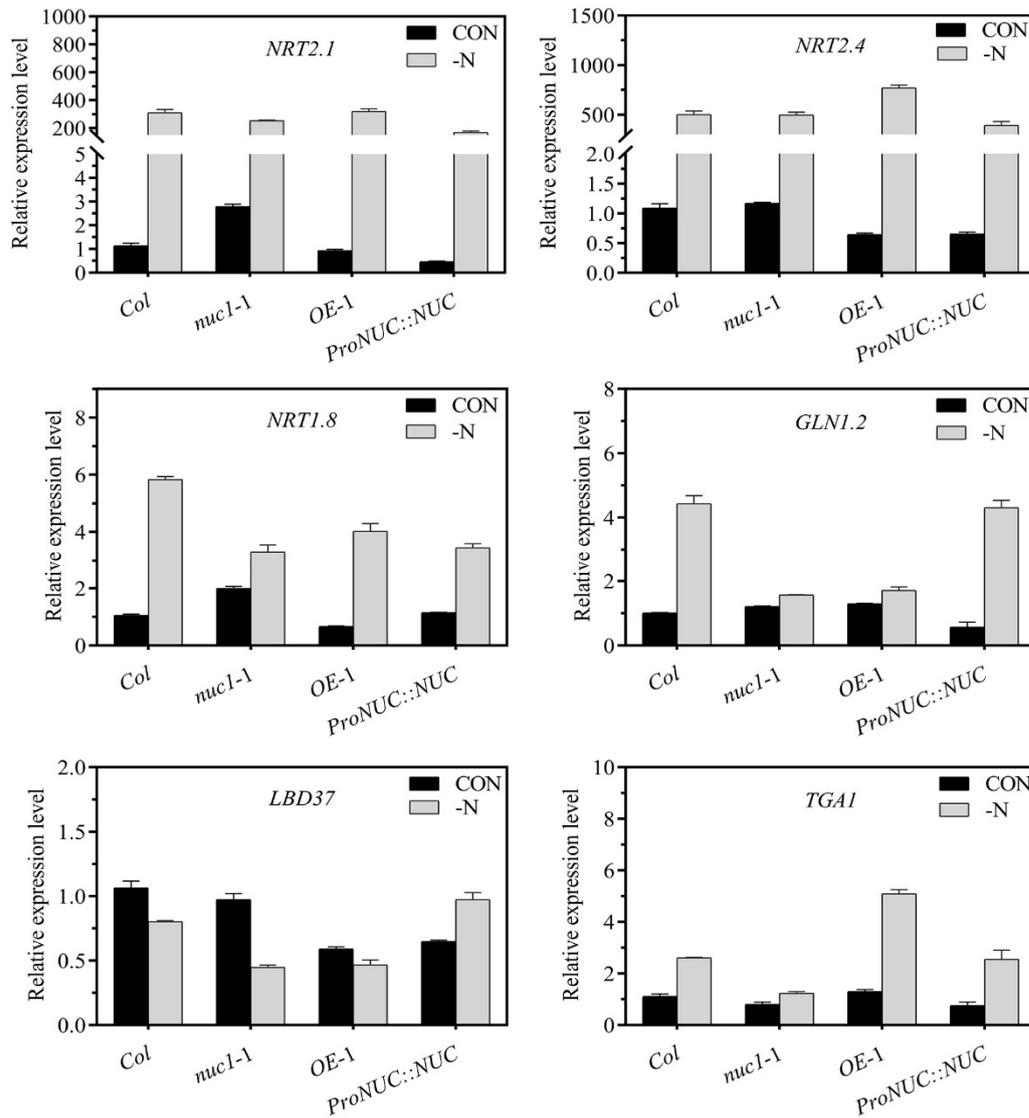


Figure S3. The lateral root phenotype of the 10-day-old seedlings, in which 4-day-old seedlings grown vertically under normal conditions were transplanted to media with (control, CON) and without (N-deficiency, -N) nitrogen for 6 days.

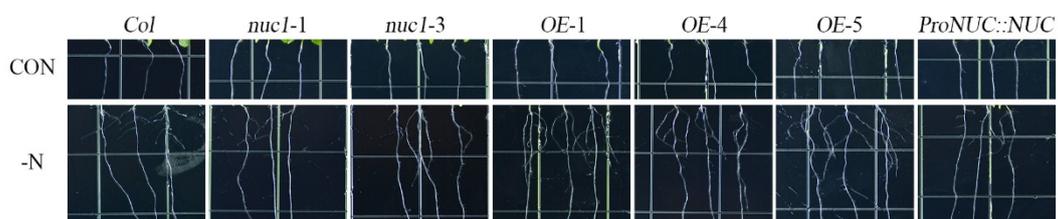


Table S1. PCR primer sequences

| Gene | Forward primer | Reverse primer |
|---------|-----------------------------------|--------------------------------|
| Tubulin | TGTGGAGAATGCAGATGAGT | ACCATGAAGAAGTGGAGACG |
| NUC | TACAGAATTACCCTTGAAAACG | CAAATCCATCCATTGATAGACG |
| NRT2.1 | TGGGAGTTGAGTTGAGCACTGA | GCCATTCCGAAAACATGCTGCTA |
| NRT2.4 | GGAAGATGAAGTGAAGTCTAC | CACGCTCCGATCTACTATT |
| NRT1.8 | GGCTTCAGATTCTTGATAG | AACCACAGAGTAGAGGATGG |
| GLN1.2 | TGTGATGCTTACACTCCAG | GCCAGTTCACATCCTTCT |
| LBD37 | AACACCTTCTTACACGG | TTCTCCTCCTCCGTTACC |
| TGA1 | CCACCGAGAAGGTTTGAA | CTGACAAGCTGTCTGGTT |
| NUC-CDs | CGGAATTCATGACAAGTGAAGTTCTCAA | CGGGATCCTCAAATCCATCCATTGATAGAC |
| NUC-P | CCTTAATTA AGAATCAGTTTAAGAGTTGTAGG | GGACCGGTGTCTTCTTCTGGGTTCTTGA |

Table S2. The nitrate-responsive cis-element (NRE) of NUC

| Gene | Chr | start | end | strand | score | p-val | sequence |
|------|------|-------|------|--------|----------|----------|---------------------------------------------|
| NUC | Chr5 | 219 | 261 | - | -20.9152 | 1.52E-05 | TGAAAGAGGTAAAACAGATCCAACGGTTATGGTCACATCTCTT |
| NUC | Chr5 | 1513 | 1555 | + | -26.697 | 7.42E-05 | GCAAACATAGGGTGCCTATAAAGTAATCAAGGGATGTTAAATT |

Table S3. NUC binding motifs in the promoters of genes

| Gene | Chr | Start | End | Strand | Score | p-value | Function in planta |
|---------------|------|-------|------|--------|---------|----------|-----------------------------------------------|
| <i>NRT2.1</i> | Chr1 | 512 | 519 | + | 14.9455 | 1.79E-05 | High-affinity nitrate transporter |
| <i>NRT2.4</i> | Chr5 | 579 | 586 | - | 14.9455 | 1.79E-05 | High-affinity nitrate transporter |
| <i>NRT1.8</i> | Chr4 | 324 | 330 | - | 13.1818 | 6.51E-05 | Nitrate retrieval from xylem |
| <i>GLN1.2</i> | Chr1 | 865 | 872 | + | 14.9455 | 1.79E-05 | Nitrogen assimilation |
| <i>LBD37</i> | Chr5 | 989 | 996 | + | 14.9455 | 1.79E-05 | Negative regulator of nitrate-related genes |
| <i>TGA1</i> | Chr5 | 1787 | 1794 | + | 14.9455 | 1.79E-05 | Positive regulator of root system development |

Table S4. MGP binding motifs in the promoters of genes

| Gene | Chr | Start | End | Strand | Score | p-value | Function in planta |
|---------------|------|-------|------|--------|---------|----------|-------------------------------------------------------------------------|
| <i>NRT1.2</i> | Chr1 | 179 | 184 | + | 11.1394 | 2.89E-04 | Nitrate uptake in roots and ABA-mediated inhibition of seed germination |
| <i>NRT1.2</i> | Chr1 | 485 | 490 | + | 11.1394 | 2.89E-04 | |
| <i>NRT1.2</i> | Chr1 | 770 | 775 | - | 11.1394 | 2.89E-04 | |
| <i>NRT1.7</i> | Chr1 | 242 | 247 | - | 11.1394 | 2.89E-04 | Nitrate remobilization from old to young leaves |
| <i>NRT2.1</i> | Chr1 | 628 | 633 | + | 11.1394 | 2.89E-04 | High-affinity nitrate transporter |
| <i>NRT2.1</i> | Chr1 | 1226 | 1231 | - | 11.1394 | 2.89E-04 | |
| <i>NRT2.2</i> | Chr1 | 665 | 670 | + | 11.1394 | 2.89E-04 | |
| <i>NRT2.2</i> | Chr1 | 1425 | 1430 | - | 11.1394 | 2.89E-04 | |
| <i>NRT2.5</i> | Chr1 | 366 | 371 | - | 11.1394 | 2.89E-04 | |
| <i>NIA1</i> | Chr1 | 444 | 449 | - | 11.1394 | 2.89E-04 | Reduced nitrate |

| | | | | | | | |
|---------------|------|------|------|---|---------|----------|--------------------------------------------------|
| <i>NIA1</i> | Chr1 | 599 | 604 | + | 11.1394 | 2.89E-04 | |
| <i>NIA1</i> | Chr1 | 888 | 893 | - | 11.1394 | 2.89E-04 | |
| <i>NIR</i> | Chr2 | 665 | 670 | - | 11.1394 | 2.89E-04 | |
| <i>NIR</i> | Chr2 | 866 | 871 | + | 11.1394 | 2.89E-04 | |
| <i>GLN1.1</i> | Chr5 | 1098 | 1103 | - | 11.1394 | 2.89E-04 | Nitrogen assimilation |
| <i>GLN1.1</i> | Chr5 | 1239 | 1244 | - | 11.1394 | 2.89E-04 | |
| <i>NLP6</i> | Chr1 | 194 | 199 | + | 11.1394 | 2.89E-04 | Master transcription factor in nitrate responses |
| <i>NLP6</i> | Chr1 | 321 | 326 | + | 11.1394 | 2.89E-04 | |
| <i>NLP6</i> | Chr1 | 1666 | 1671 | + | 11.1394 | 2.89E-04 | |
| <i>NLP6</i> | Chr1 | 1839 | 1844 | + | 11.1394 | 2.89E-04 | |
| <i>NLP6</i> | Chr1 | 1967 | 1972 | + | 11.1394 | 2.89E-04 | |
| <i>NLP7</i> | Chr4 | 1267 | 1272 | + | 11.1394 | 2.89E-04 | |
| <i>TCP20</i> | Chr3 | 1714 | 1719 | - | 11.1394 | 2.89E-04 | Positive regulator of nitrogen metabolism |
| <i>LBD37</i> | Chr5 | 558 | 563 | - | 11.1394 | 2.89E-04 | Negative regulator of nitrate-related genes |
| <i>LBD37</i> | Chr5 | 1820 | 1825 | + | 11.1394 | 2.89E-04 | |
| <i>LBD37</i> | Chr5 | 1820 | 1825 | + | 11.1394 | 2.89E-04 | |
| <i>LBD38</i> | Chr5 | 1841 | 1846 | + | 11.1394 | 2.89E-04 | |
| <i>LBD39</i> | Chr5 | 1264 | 1269 | + | 11.1394 | 2.89E-04 | |
| <i>TGA1</i> | Chr5 | 660 | 665 | - | 11.1394 | 2.89E-04 | Positive regulator of root system development |