Supplementary Information

Shoot-to-root mobile CEPD-like 2 integrates shoot nitrogen status to systemically regulate nitrate uptake in *Arabidopsis*

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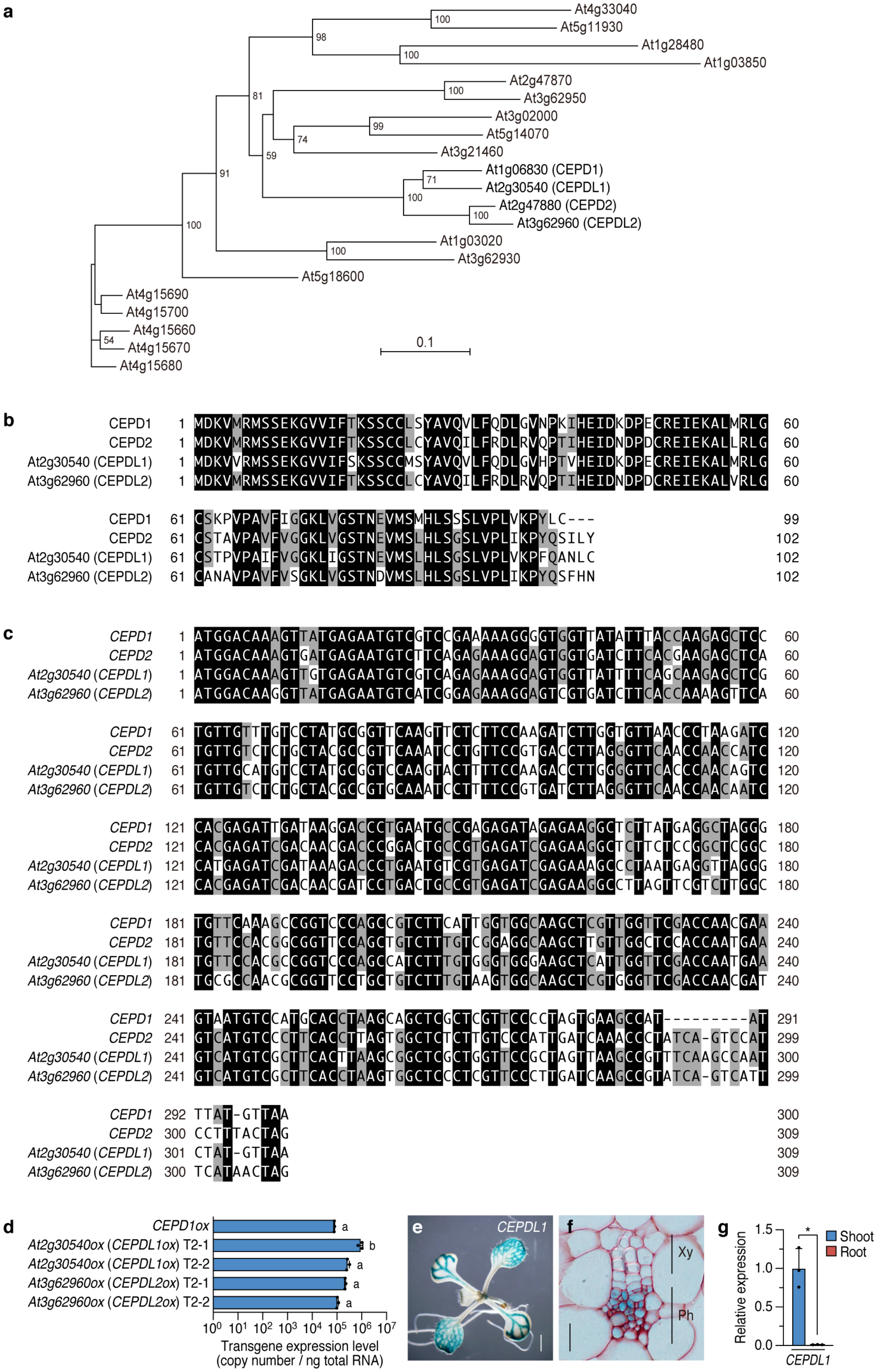

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CEPD1                1 MDKVMRMSSEKGVVIFTKSSCCLSYAVQVLFQDLGVNPKIHEIDKDPECREIEKALMRLG  60
CEPD2                1 MDKVMRMSSEKGVVIFTKSSCCLCYAVQILFRDLRVQPTIHEIDNDPDCREIEKALLRLG  60
At2g30540 (CEPDL1)   1 MDKVVRMSSEKGVVIFSKSSCCMSYAVQVLFQDLGVHPTVHEIDKDPECREIEKALMRLG  60
At3g62960 (CEPDL2)   1 MDKVMRMSSEKGVVIFTKSSCCLCYAVQILFRDLRVQPTIHEIDNDPDCREIEKALVRLG  60

CEPD1               61 CSKPVPAVFIGGKLVGSTNEVMSMHLSSSLVPLVKPYLC---                    99
CEPD2               61 CSTAVPAVFVGGKLVGSTNEVMSLHLSGSLVPLIKPYQSILY                   102
At2g30540 (CEPDL1)  61 CSTPVPAIFVGGKLIGSTNEVMSLHLSGSLVPLVKPFQANLC                   102
At3g62960 (CEPDL2)  61 CANAVPAVFVSGKLVGSTNDVMSLHLSGSLVPLIKPYQSFHN                   102
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CEPD1                1 ATGGACAAAGTTATGAGAATGTCGTCCGAAAAAGGGGTGGTTATATTTACCAAGAGCTCC  60
CEPD2                1 ATGGACAAAGTGATGAGAATGTCTTCAGAGAAAGGAGTGGTGATCTTCACGAAGAGCTCA  60
At2g30540 (CEPDL1)   1 ATGGACAAAGTTGTGAGAATGTCGTCAGAGAAAGGAGTGGTTATTTTCAGCAAGAGCTCG  60
At3g62960 (CEPDL2)   1 ATGGACAAGGTTATGAGAATGTCATCGGAGAAAGGAGTCGTGATCTTCACCAAAAGTTCA  60

CEPD1               61 TGTTGTTTGTCCTATGCGGTTCAAGTTCTCTTCCAAGATCTTGGTGTTAACCCTAAGATC 120
CEPD2               61 TGTTGTCTCTGCTACGCCGTTCAAATCCTGTTCCGTGACCTTAGGGTTCAACCAACCATC 120
At2g30540 (CEPDL1)  61 TGTTGCATGTCCTATGCGGTCCAAGTACTTTTCCAAGACCTTGGGGTTCACCCAACAGTC 120
At3g62960 (CEPDL2)  61 TGTTGTCTCTGCTACGCCGTGCAAATCCTTTTCCGTGATCTTAGGGTTCAACCAACAATC 120

CEPD1              121 CACGAGATTGATAAGGACCCTGAATGCCGAGAGATAGAGAAGGCTCTTATGAGGCTAGGG 180
CEPD2              121 CACGAGATCGACAACGACCCGGACTGCCGTGAGATCGAGAAGGCTCTTCTCCGGCTCGGC 180
At2g30540 (CEPDL1) 121 CATGAGATCGATAAAGACCCTGAATGTCGTGAGATCGAGAAAGCCCTAATGAGGTTAGGG 180
At3g62960 (CEPDL2) 121 CACGAGATCGACAACGATCCTGACTGCCGTGAGATCGAGAAGGCCTTAGTTCGTCTTGGC 180

CEPD1              181 TGTTCAAAGCCGGTCCCAGCCGTCTTCATTGGTGGCAAGCTCGTTGGTTCGACCAACGAA 240
CEPD2              181 TGTTCCACGGCGGTTCCAGCTGTCTTTGTCGGAGGCAAGCTTGTTGGCTCCACCAATGAA 240
At2g30540 (CEPDL1) 181 TGTTCCACGCCGGTCCCAGCCATCTTTGTGGGTGGGAAGCTCATTGGTTCGACCAATGAA 240
At3g62960 (CEPDL2) 181 TGCGCCAACGCGGTTCCTGCTGTCTTTGTAAGTGGCAAGCTCGTGGGTTCGACCAACGAT 240

CEPD1              241 GTAATGTCCATGCACCTAAGCAGCTCGCTCGTTCCCCTAGTGAAGCCAT---------AT 291
CEPD2              241 GTCATGTCCCTTCACCTTAGTGGCTCTCTTGTCCCATTGATCAAACCCTATCA-GTCCAT 299
At2g30540 (CEPDL1) 241 GTCATGTCGCTTCACTTAAGCGGCTCGCTGGTTCCGCTAGTTAAGCCGTTTCAAGCCAAT 300
At3g62960 (CEPDL2) 241 GTCATGTCGCTTCACCTAAGTGGCTCCCTCGTTCCCTTGATCAAGCCGTATCA-GTCATT 299

CEPD1              292 TTAT-GTTAA                                                   300
CEPD2              300 CCTTTACTAG                                                   309
At2g30540 (CEPDL1) 301 CTAT-GTTAA                                                   309
At3g62960 (CEPDL2) 300 TCATAACTAG                                                   309
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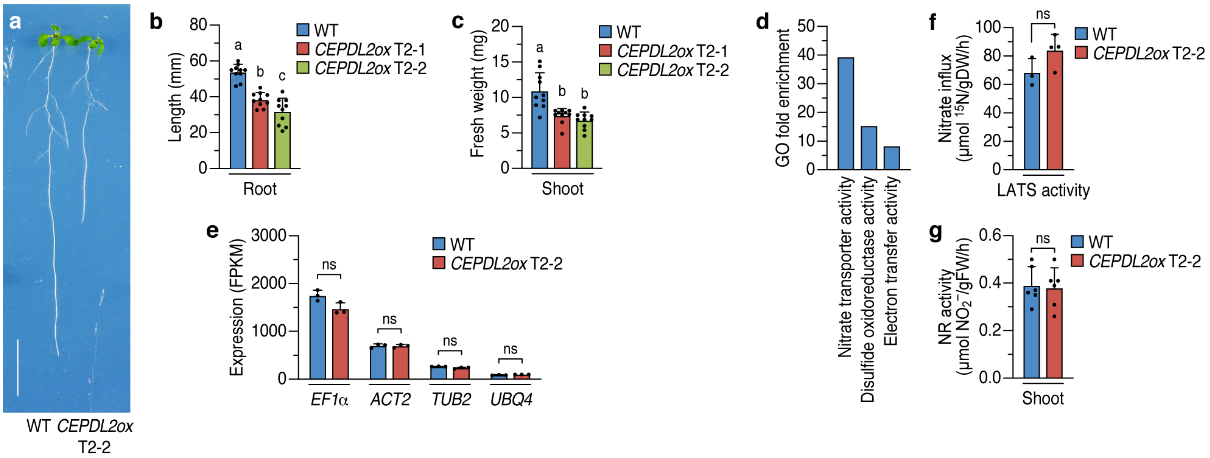
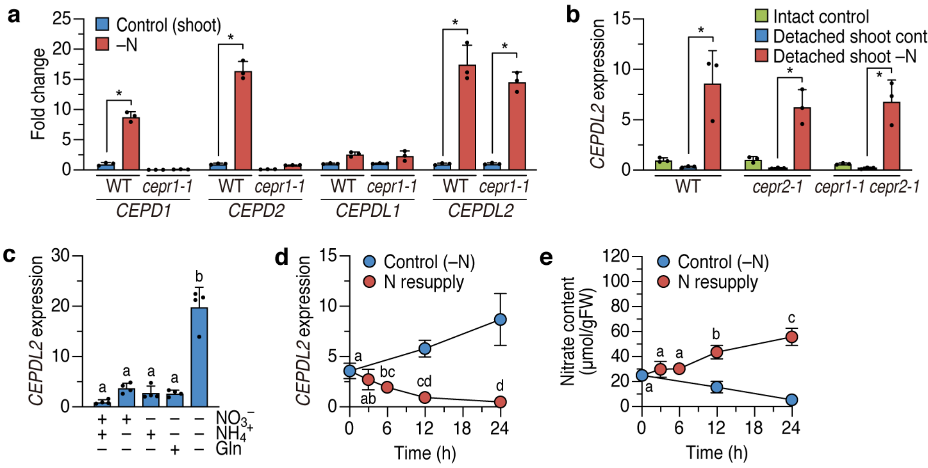
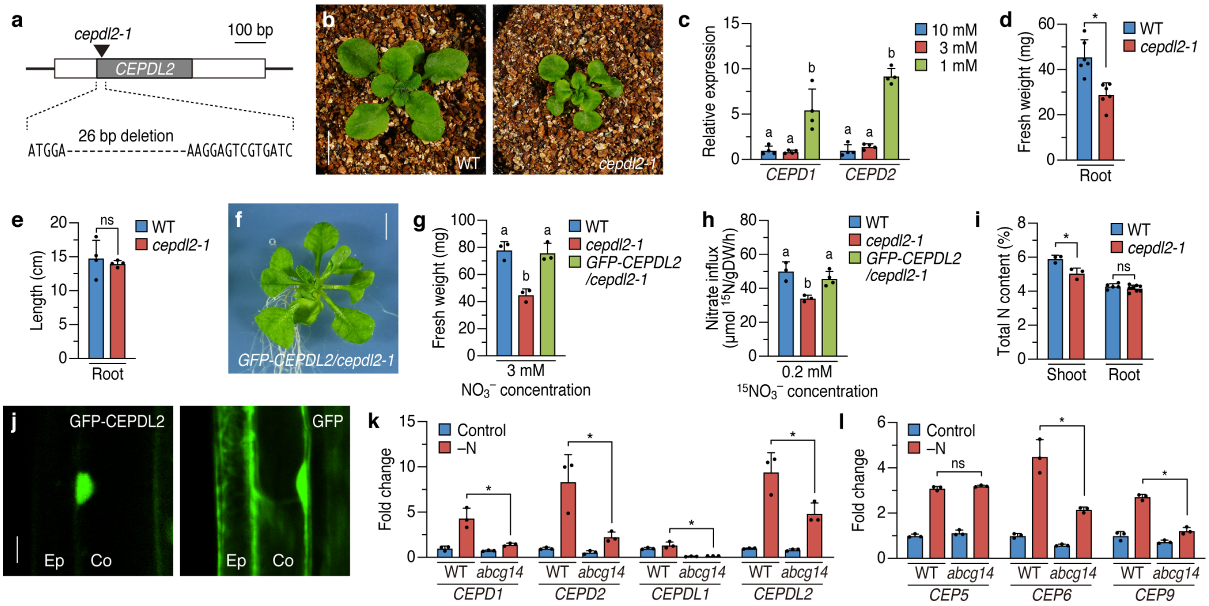
Supplementary Fig. 1 At2g30540 and At3g62960 upregulate the expression of *NRT2.1* when overexpressed. a Phylogenetic tree of the class III glutaredoxin family proteins. Protein sequences were aligned using ClustalW and the tree was generated with the neighbor-joining method. Bootstrap values (above 50%) from 1000 replicates are indicated at each node. **b** Multiple sequence alignment of At2g30540 (CEPDL1), At3g62960 (CEPDL2) and CEPD1/2 polypeptide amino acid sequences. Identical residues are boxed in black, and similar residues are boxed in gray. **c** Nucleotide sequence alignment of *CEPD/CEPDL* family genes. **d** Absolute quantification of transgene transcripts by qRT-PCR analysis of the roots of 10-day-old transgenic plants overexpressing the indicated gene and grown on N-replete medium. Different letters indicate statistically significant differences ($P < 0.05$, two-tailed one-way ANOVA, $n = 3$). **e** Histochemical staining of 10-day-old seedlings transformed with the *CEPDL1pro:GUS* gene. Scale bar = 1 mm. **f** Cross-section of the leaf vascular tissues pictured in ‘e’. Scale bar = 10 µm. **g** qRT-PCR analysis of *CEPDL1* transcripts in the shoots and roots of 7-day-old WT plants cultured on 10 mM NO_3^- condition (mean ± SD, $*P < 0.05$ by two-tailed non-paired Student's *t* test, $n = 3$).



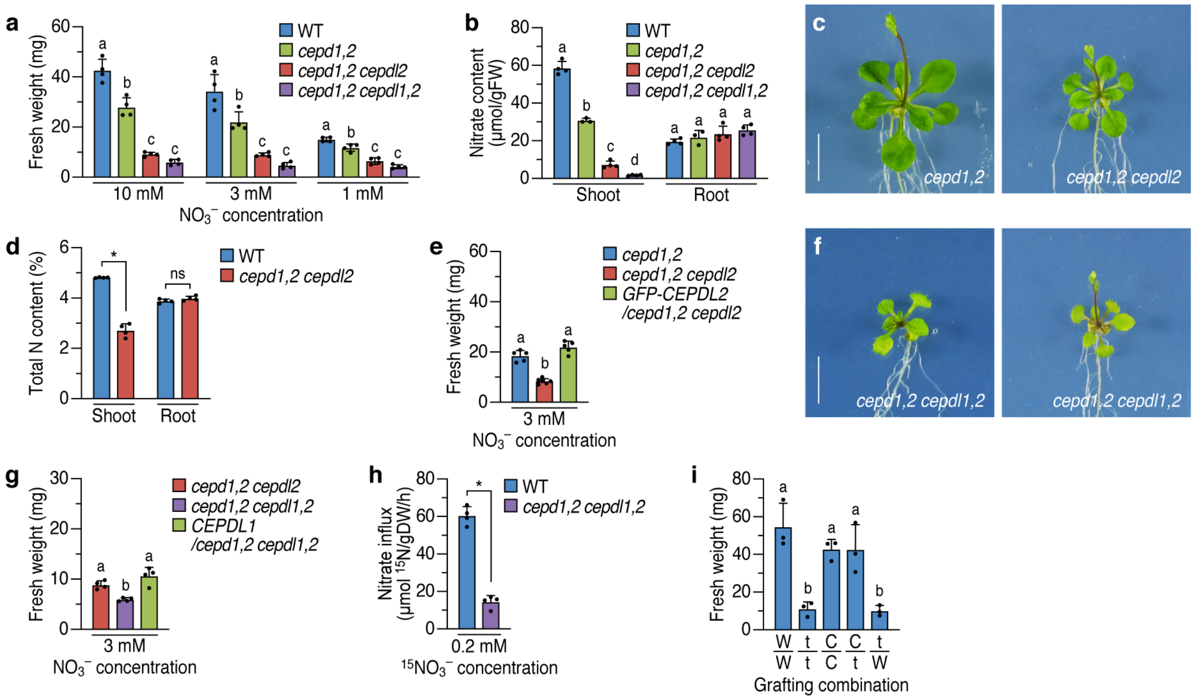
Supplementary Fig. 2 Phenotypes of *CEPDL2ox* plants. a Phenotypes of 10-day-old WT and *CEPDL2ox* plants grown under 10 mM NO$_3^-$ condition. Scale bar = 1 cm. **b** Comparison of root length between 10-day-old WT and *CEPDL2ox* plants (n = 10). **c** Comparison of shoot fresh weight between 14-day-old WT and *CEPDL2ox* plants (n = 10). **d** Gene ontology analysis of the top 150 upregulated genes in *CEPDL2ox* plants. **e** Expression stability of representative reference genes in roots of 10-day-old WT and *CEPDL2ox* plants (n = 3). False discovery rate (FDR) q < 0.05 was considered significant. **f** Calculated LATS activity of the roots of *CEPDL2ox* plants (n = 4). **g** Nitrate reductase (NR) activity in shoots of 17-day-old WT and *CEPDL2ox* plants (n = 6).



Supplementary Fig. 3 *CEPDL2* expression is regulated by the shoot N status. a qRT-PCR analysis of *CEPD1/2* and *CEPDL1/2* transcripts in the leaves of 12-day-old WT and *cepr1-1* plants after N starvation for 24 h (n = 3). **b** qRT-PCR analysis of *CEPDL2* transcripts in detached leaves of WT, *cepr2-1* and *cepr1-1 cepr2-1* plants after N starvation for 24 h (n = 3). **c** qRT-PCR analysis of *CEPDL2* transcripts in detached WT leaves treated with various N sources for 24 h (n = 3). **d** Changes in *CEPDL2* transcript levels in WT leaves subjected to N starvation for 24 h, followed by continued N starvation (-N) or N resupply (NH_4^+ 10 mM, NO_3^- 10 mM) (n = 3). **e** Changes in nitrate content in WT shoots subjected to N starvation for 24 h, followed by continued N starvation (-N) or N resupply (n = 3).



Supplementary Fig. 4 Loss of *CEPDL2* impairs nitrate acquisition. a Schematic representation of the deletion site in *cepdl2-1*. **b** Phenotypes of 4-week-old WT and *cepdl2-1* plants grown in vermiculite with nutrient solutions containing 3 mM NO_3^-. Scale bar = 1 cm. **c** Levels of *CEPD1* and *CEPD2* transcripts in WT shoots grown under various nitrate conditions for 21 d (n = 3). **d** Fresh weight of roots of WT and *cepdl2-1* plants grown under 3 mM NO_3^- conditions for 21 d (n = 6). **e** Root length of WT and *cepdl2-1* plants at 21 d (n = 6). **f** Phenotype of 21-day-old *cepdl2-1* mutant plants complemented with *GFP-CEPDL2* under 3 mM NO_3^- conditions. Scale bar = 5 mm. **g** Fresh weight of shoots of 21-day-old *cepdl2-1* plants complemented with the *GFP-CEPDL2* (n = 3). **h** Recovery of HATS activity in complemented plants. **i** Total N content in shoots and roots of WT and *cepdl2-1* plants grown under 3 mM NO_3^- condition for 21 d. **j** Comparison of GFP signals in the root cortex region of 9-day-old plants expressing GFP-CEPDL2 under the *CEPDL2* promoter or free GFP under the *NRT2.1* promoter. Scale bar = 10 µm. **k** qRT-PCR analysis of CEPD1/2 and CEPDL1/2 transcripts in the shoots of 14-day-old WT and *abcg14* plants after N starvation for 24 h (n = 3). **l** qRT-PCR analysis of *CEP* transcripts in the roots of 14-day-old WT and *abcg14* plants after N starvation for 24 h (n = 3).



Supplementary Fig. 5 The CEPDL2 and CEPD1/2 systemically regulate root nitrate acquisition. **a** Fresh weight of shoots of WT and multiple-mutant plants grown under various NO$_3^-$ conditions for 17 d (n = 4). **b** Nitrate content in shoots and roots of WT and mutant plants grown under 3 mM NO$_3^-$ conditions (n = 4). **c** Early flowering phenotypes of 21-day-old multiple-mutant plants grown on 3 mM NO$_3^-$ medium. Scale bar = 5 mm. **d** Total N content in shoots and roots of WT and *cepd1,2 cepdl2* triple-mutant plants grown under 3 mM NO$_3^-$ conditions for 17 d (n = 4). **e** Fresh weight of shoots of 17-day-old *cepd1,2 cepdl2* plants complemented with the *GFP-CEPDL2* construct (n = 5). **f** Phenotype of 17-day-old *cepd1/2 cepdl1/2* quadruple mutant plants grown on 3 mM NO$_3^-$ medium (left). Early flowering phenotype of 21-day-old quadruple-mutant plants (right). Scale bar = 5 mm. **g** Fresh weight of shoots of 17-day-old *cepd1/2 cepdl1/2* quadruple-mutant plants complemented with the *CEPDL1* genomic fragment (n = 4). **h** HATS activity in *cepd1/2 cepdl1/2* quadruple-mutant roots (n = 4). **i** Fresh weight of shoots of reciprocally grafted plants (n = 3). Abbreviations: W, wild-type; t, *cepd1,2 cepdl2* triple mutant; C, *cepd1,2 cepdl2* triple mutant complemented with *GFP-CEPDL2*.