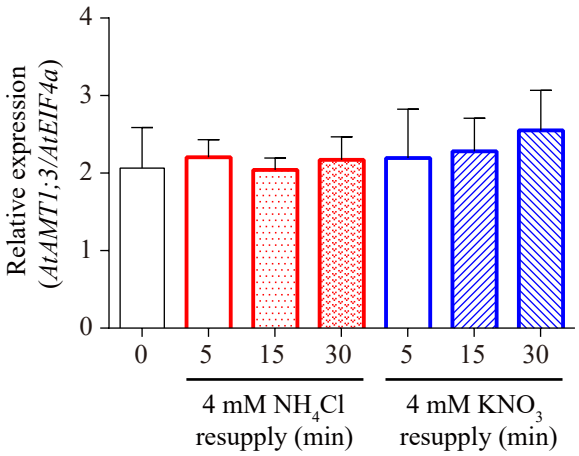
**Supplemental Table S1: Primers used in this study.**

| Primer | Sequence (5' to 3') | Remark |
|---|---|---|
| qAtUBQ10_F | CTTCGTCAAGACTTTGACCG | Q-PCR |
| qAtUBQ10_R | CTTCTTAAGCATAACAGAGACGAG | |
| qAtAMT13_F | GAAGGCCATATGGACTATTTATGGG | Q-PCR |
| qAtAMT13_R | CGAGGAGGAGTAGCTGATCGAGG | |
| qAtEIF4a_F | TCATAGATCTGGTCCTTGAAACC | Q-PCR |
| qAtEIF4a_R | GGCAGTCTCTTCGTGCTGAC | |
| p426-AtAMT13-EcoR I_F | CGGAATTCATGTCAGGAGCAATAACAT | AtAMT1;3 cloning |
| p426-AtAMT13-EcoR I_R | CGGAATTCTTAAACGCGAGGAGGAGTAG | |
| p426-T464A_F | GATGGATATGGCACGTCACGG | AtAMT1;3 T464As |
| p426-T464A_R | CCTTGCATTTCATGCTGC | |
| p426-S480A_F | TGATGATGAGGCTCATAGAGTG | AtAMT1;3 S480As |
| p426-S480A_R | TTATCATGGTAGATATAAGCAAAG | |
| p426-S480D_F | TGATGATGAGGATCATAGAGTGGATC | AtAMT1;3 S480D |
| p426-S480D_R | TTATCATGGTAGATATAAGCAAAG | |
| p426-S487A_F | GGATCCTGGAGCTCCTTTCCC | AtAMT1;3 S487As |
| p426-S487A_R | ACTCTATGAGACTCATCATCATTATC | |
| p426-S487D_F | GGATCCTGGAGATCCTTTCCCTC | AtAMT1;3 S487D |
| p426-S487D_R | ACTCTATGAGACTCATCATC | |
| p426-T494A/T464AT494A_F | TCGATCAGCTGCTCCTCCTCG | AtAMT1;3 T494A/T464A T494A |
| p426-T494A/T464AT494A_R | GGGAAAGGAGATCCAGGATC | |
| p426-T494D/T464AT494D_F | TCGATCAGCTGATCCTCCTCGC | AtAMT1;3 T494D/T464A T494D |
| p426-T494D/T464AT494D_R | GGGAAAGGAGATCCAGGA | |
| p426-T464D_F | GATGGATATGGATCGTCACGGTGG | AtAMT1;3 T464D/T464D T494A/T464D T494D |
| p426-T464D_R | CCTTGCATTTCATGCTGC | |
| p426-H474stop_F | TTATATCTACTAAGATAATGATGATGAGTCTC ATAGAGTGG | AtAMT1;3 H474stop |
| p426-H474stop_R | GCAAAGCCACCGTGACGT | |
| AtAMT1;3pro-Xba I | GCTCTAGAGTTGCAAGAATATTAATAGAAAT AC | AtAMT1;3 promoter cloning |
| AtAMT1;3pro-Apa I | GCGGGCCCGTTTGAGAGAGCTGAGAGAGA GAAAGA | |
| pT-AtAMT1;3pro-AtAMT1;3_F | GCGGGCCCATGTCAGGAGCAATAACATGC | AtAMT1;3/T464A/T494A/T464A T494A |
| pT-AtAMT1;3pro-AtAMT1;3/T464A/T494A/T464A T494A_R | GCGGGCCCTTAAACGCGAGGAGGAG | |
| pT-AtAMT1;3pro-AtAMT1;3_F | GCGGGCCCATGTCAGGAGCAATAACATGC | AtAMT1;3 T494D/T464A T494D |
| pT-AtAMT1;3pro-AtAMT1;3 T494D/T464AT494D_R | GCGGGCCCTTAAACGCGAGGAGGAT | |
| poo2-AtAMT1;3_F | GGACTAGTATGTCAGGAGCAATAACATGC | AtAMT1;3/T464A/T494A/T464A/T494D |
| poo2-AtAMT1;3/T494A_R | CGGAATTCTTAAACGCGAGGAGGAG | |
| poo2-AtAMT1;3_F | GGACTAGTATGTCAGGAGCAATAACATGC | AtAMT1;3 T494D/T464A T494D |
| poo2-AtAMT1;3 T494D_R | CGGAATTCTTAAACGCGAGGAGGAT | |

**Supplemental Table S2: The phosphorylation sites in AtAMT1s C-terminals.**

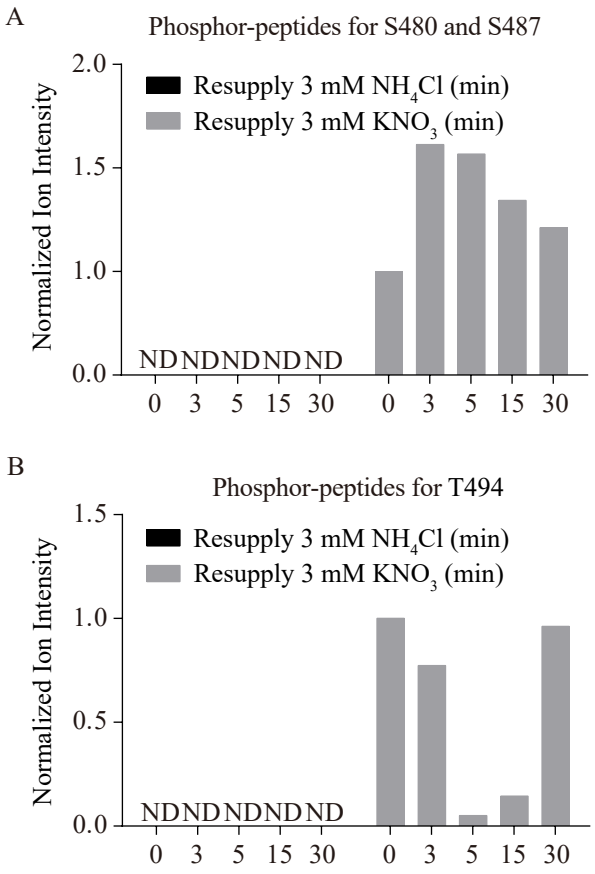
| Gene | Phosphorylation Site | Treatment | Background | Tissue | Detection | Refereences |
|---|---|---|---|---|---|---|
| AtAMT1;1 | T460 | flg22 | *col-0* | suspension cells | LC-MS/MS (IMAC) | Nühse et al., 2004 |
| | T460, S475, S488, S492, T496 | flg22 | *col-0* | suspension cells | HPLC-MS/MS ($^{15}$N) | Benschop et al., 2007 |
| | S475 | - | *landsberg erecta* | suspension cells | LC-MS/MS (IMAC) | Sugiyama et al., 2008 |
| | T460 | - | *columbia* | leaves | LC-MS/MS (IMAC) | Whiteman et al., 2008 |
| | T460 | - | *col-0* | protoplasts/seedlings | LC-MS/MS | Jones et al., 2009 |
| | T460, S475, S488, S490 | -N/ammonium resupply | *col-0* | roots | HPLC-MS/MS ($^{15}$N) | Lanquar et al., 2009 |
| | S488, S490, S492, T496, T497, T499 | end of night/end of day | *col-0* | shoots/rosette leaves | LC-MS/MS ($TiO_2$) | Reiland et al., 2009 |
| | S490 | ABA, GA, JA, IAA or cytokinin | *col-0* | suspension cells | MS/MS ($TiO_2$) | Chen et al., 2010 |
| | S488 | ABA | *Lehle* | seedlings | MS/MS ($^{15}$N) | Kline et al., 2010 |
| | T460, S475 | - | *landsberg erecta* | suspension cells | LC-MS/MS (IMAC) | Nakagami et al., 2010 |
| | T460, S475, S488, S490, S492, T497 | -N/ammonium resupply or –N/nitrate resupply | *col-0* | seedlings | LC-MS/MS ($TiO_2$) | Engelsberger and Schulze, 2012 |
| | T460 | -N/ammonium resupply | *col-0* | roots | pT464 antibody | Lanquar et al., 2009 |
| | T460 | -N/ammonium resupply | *col-0* | roots | GMDMT(p)RAGGFA antibody | Straub et al., 2017 |
| AtAMT1;2 | T472 | - | *col-0* | protoplasts/seedlings | LC-MS/MS | Jones et al., 2009 |
| | T472 | -N/ammonium resupply | *col-0* | roots | GMDMT(p)RAGGFA antibody | Straub et al., 2017 |
| AtAMT1;3 | S480, S487, T494 | -N/ammonium resupply or –N/nitrate resupply | *col-0* | seedlings | LC-MS/MS ($TiO_2$) | Engelsberger and Schulze, 2012 |
| | T464, S487 | ammonium or nitrate depletion | *col-0* | roots | LC-MS/MS ($TiO_2$) | Menz et al., 2016 |

Note: -, untreatment; -N, nitrogen starvation.



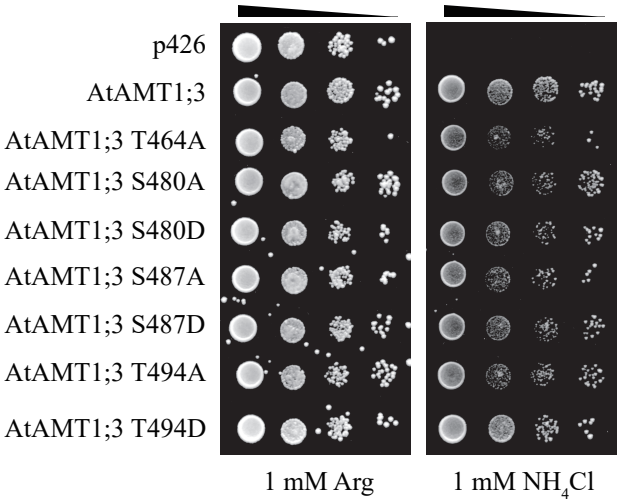

**Supplemental Fig. S1. Transcript expression levels of *AtAMT1;3* in *qko+13* roots in response to ammonium and nitrate resupply.**

Transcript expression levels of *AtAMT1;3* in roots quantified by qPCR with three replicates, and normalized by *AtEIF4a* expression level. Six-weeks-old hydroponically-grown Arabidopsis mutant *qko+13* (*atamt1;1*, *atamt1;2* and *atamt2;1*) were subjected to N starvation for 4 days, and then resupplied with 4 mM $NH_4Cl$ or 4 mM $KNO_3$ for 5, 15 and 30 min. Bars indicate means ± SD (n=3) and significant differences at $P<0.001$ according to Tukey's test are indicated by different letters.



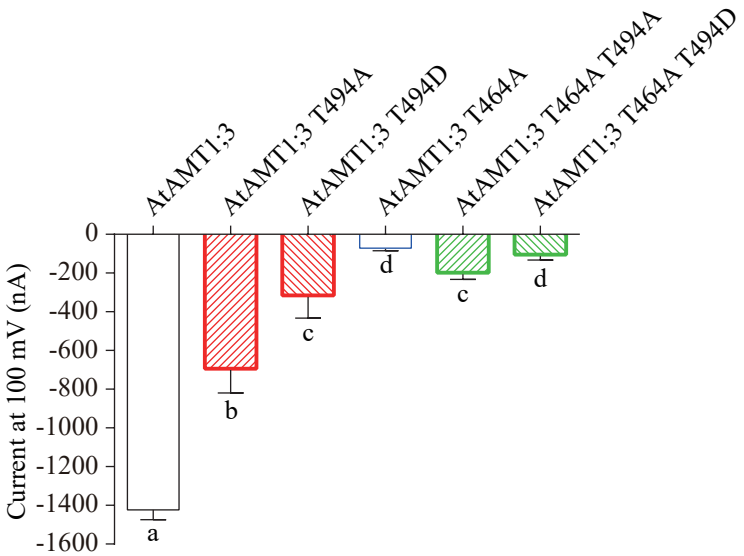

**Supplemental Fig. S2. Phosphorylation dynamics of AtAMT1;3 CTR$^{NC}$ at multiple sites (S480, S487, and T494) in response to nitrate or ammonium.**

Normalized ion intensity of (A) phosphorylated AtAMT1;3 peptides HGGFAYIYHDND DES(ph)HRVDPGS(ph)PFPR (S480/S487) and (B) SAT(ph)PPRV (T494) that responded to nitrate or ammonium. The phosphorylated peptides were identified by phosphoproteomics, and the data was obtained from Engelsberger and Schulze (2012). ND, not determined.

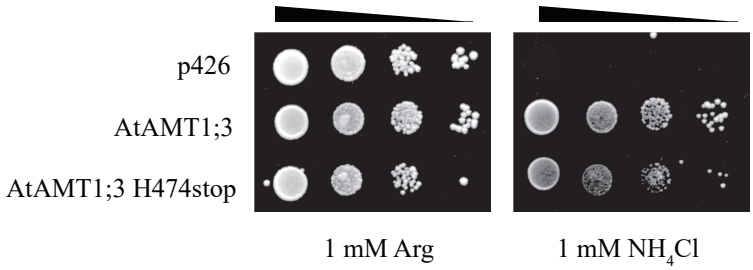



**Supplemental Fig. S3. Yeast growth complementation of AtAMT1;3 S480, S487 and T494 single phosphor-mutants.**

The yeast mutant *31019b* (*Δmep1-3*) were transformed with empty vector *p426*, AtAMT1;3 single phosphor-variant T464A, S480A, S480D, S487A, S487D, T494A or T494D. Transformants were selected on YNB medium supplemented with 1 mM arginine (Arg). Five microliters of yeast cell suspensions from overnight cultures were spotted in 1- to 4-fold dilution on YNB medium supplemented with either 1 mM Arg or 1 mM ammonium as the sole N source at pH 5.5 for 3 days at 28℃.

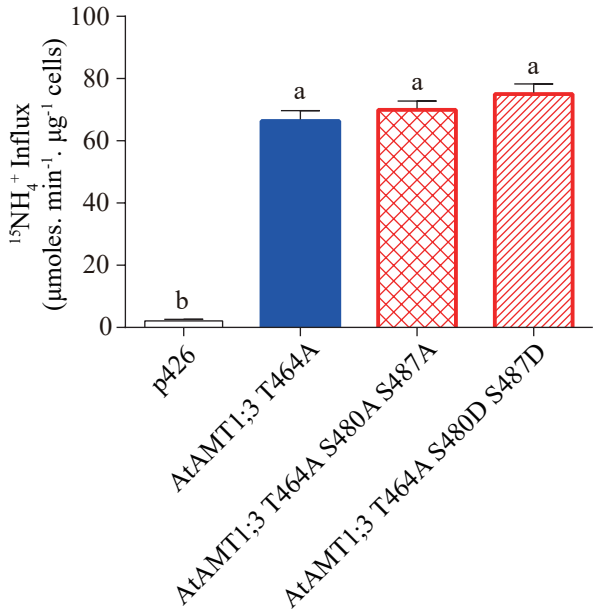



**Supplemental Fig. S4. Functional characterization of AtAMT1;3 T494 single phosphor-mutants and T464 T494 double phosphor-mutants in *Xenopus* oocytes.** Ammonium induced currents in oocytes injected with AtAMT1;3, and AtAMT1;3 single phosphor-mutants T494A, T494D or T464A, and double phosphor-mutants T464A T494D or T464A T494D. Net currents were measured conditions of 1000 μM $NH_4Cl$ at -100 mV. Bars indicate means ± SD (n≥4) and significant differences at $P<0.05$ according to Tukey's test are indicated by different letters.




**Supplemental Fig. S5. Yeast growth complementation of AtAMT1;3 CTR$^{NC}$ deletion mutant.**

The yeast mutant *31019b* (*Δmep1-3*) were transformed with empty vector *p426*, AtAMT1;3 wild type or the CTR$^{NC}$ deletion mutant AtAMT1;3 H474stop. Transformants were selected on YNB medium supplemented with 1 mM arginine (Arg). Five microliters of yeast cell suspensions from overnight cultures were spotted in 1- to 4-fold dilution on YNB medium supplemented with either 1 mM Arg or 1 mM ammonium as the sole N source at pH 5.5 for 3 days at 28℃.




**Supplemental Fig. S6. Functional characterization of AtAMT1;3 T464 S480 S487 triple phosphor-mutants in yeasts.**

Influx of $^{15}N$-labelled ammonium into yeast mutant *31019b* expressing empty vector *p426*, AtAMT1;3 single phosphor-variant T464A and triple phosphor-variants T464A S480A S487A or T464A S480D S487D. The yeast cells were grown on YNB medium supplemented with 3% glucose and 1 mM Arg to OD600 of 0.8. Cells were harvested and suspended in 100 mM potassium phosphate buffer (pH 5.8) to a final OD600 of 40 for $^{15}N$-labelled ammonium influx assay. Influx assays were performed at the concentration of 250 µM of $^{15}NH_4^+$ for 6 min. Bars indicate means ± SD (n=5) and significant differences at $P<0.001$ according to Tukey's test are indicated by different letters.